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(54) Title: CYTOKINE-, STRESS-, AND ONCOPROTEIN-ACTIVATED HUMAN PROTEIN KINASE KINASES (57) Abstract <p>Disclosed are human mitogen-activated (MAP) kinase kinase isoforms (MKKs). MKKs mediate unique signal transduction pathways that activate human MAP kinases p38 and JNK, which result in activation of other factors, including activating transcription factor-2 (ATF2) and c-Jun. The pathways are activated by a number of factors, including cytokines and environmental stress. Methods are provided for identifying reagents that modulate MKK function or activity and for the use of such reagents in the treatment of MKK-mediated disorders.</p>		

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CYTOKINE-, STRESS-, AND ONCOPROTEIN-ACTIVATEDHUMAN PROTEIN KINASE KINASESBackground of the Invention

5 This invention relates to protein kinases.

Mitogen-activated protein (MAP) kinases are important mediators of signal transduction from the cell surface to the nucleus. Multiple MAP kinases have been described in yeast including SMK1, HOG1, NPK1, FUS3, and
10 KSS1. In mammals, the MAP kinases identified are extracellular signal-regulated MAP kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38 kinase (Davis (1994) Trends Biochem. Sci. 19:470). These MAP kinase isoforms are activated by dual phosphorylation on threonine and
15 tyrosine.

Activating Transcription Factor-2 (ATF2), ATF α , and cAMP Response Element Binding Protein (CRE-BP α) are related transcription factors that bind to similar sequences located in the promoters of many genes (Ziff
20 (1990) Trends in Genet. 6:69). The binding of these transcription factors leads to increased transcriptional activity. ATF2 binds to several viral proteins, including the oncoprotein Ela (Liu and Green (1994) Nature 368:520), the hepatitis B virus X protein (Maguire
25 et al. (1991) Science 252:842), and the human T cell leukemia virus 1 tax protein (Wagner and Green (1993) Science 262:395). ATF2 also interacts with the tumor suppressor gene product Rb (Kim et al. (1992) Nature 358:331), the high mobility group protein HMG(I)Y (Du et
30 al. (1993) Cell 74:887), and the transcription factors nuclear NF- κ B (Du et al. (1993) Cell 74:887) and c-Jun (Benbrook and Jones (1990) Oncogene 5:295).

Summary of the Invention

We have identified and isolated a new group of
35 human mitogen-activated prot in kinase kinases (MKKs).

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The MKK isoforms described herein, MKK3, MKK6, and MKK4 (including MKK4- α , - β , and - γ), have serine, threonine, and tyrosine kinase activity, and specifically phosphorylate the human MAP kinase p38 at Thr¹⁸⁰ and Tyr¹⁸². The MKK4 isoforms also phosphorylate the human MAP kinases JNK (including JNK1 and JNK2) at Thr¹⁸³ and Tyr¹⁸⁵.

Accordingly, the invention features a substantially pure human MKK polypeptide having serine, threonine, and tyrosine kinase activity that specifically phosphorylates human p38 MAP kinase. MKK3 has the amino acid sequence of SEQ ID NO:2. The invention further includes MKK6 having the amino acid sequence of SEQ ID NO:4 and having serine, threonine, and tyrosine kinase activity that specifically phosphorylates human p38 MAP kinase.

The invention further features a substantially pure human MKK polypeptide having serine, threonine, and tyrosine kinase activity that specifically phosphorylates human p38 MAP kinase and JNK. MKK4 isoform MKK4- α has the amino acid sequence of SEQ ID NO:6. MKK4 isoform MKK4- β has the amino acid sequence of SEQ ID NO:8. MKK4 isoform MKK4- γ has the amino acid sequence of SEQ ID NO:10.

As used herein, the term "mitogen-activating protein kinase kinase" or "MKK" means a protein kinase which possesses the characteristic activity of phosphorylating and activating a human mitogen-activating protein kinase. Examples of MKKs include MKK3 and MKK6, which specifically phosphorylate and activate p38 MAP kinase at Thr¹⁸⁰ and Tyr¹⁸², and MKK4 isoforms which specifically phosphorylate and activate p38 MAP kinase at Thr¹⁸⁰ and Tyr¹⁸², and JNK at Thr¹⁸³ and Tyr¹⁸⁵.

The invention includes the specific p38 MKKs disclosed, as well as closely related MKKs which are

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identified and isolated by the use of probes or
antibodies prepared from the polynucleotide and amino
acid sequences disclosed for the MKKs of the invention.
This can be done using standard techniques, e.g., by
5 screening a genomic, cDNA, or combinatorial chemical
library with a probe having all or a part of the nucleic
acid sequences of the disclosed MKKs. The invention
further includes synthetic polynucleotides having all or
part of the amino acid sequence of the MKKs herein
10 described.

The term "polypeptide" means any chain of amino
acids, regardless of length or post-translational
modification (e.g., glycosylation or phosphorylation),
and includes natural proteins as well as synthetic or
15 recombinant polypeptides and peptides.

The term "substantially pure," when referring to a
polypeptide, means a polypeptide that is at least 60%, by
weight, free from the proteins and naturally-occurring
organic molecules with which it is naturally associated.
20 A substantially pure human MKK polypeptide is at least
75%, more preferably at least 90%, and most preferably at
least 99%, by weight, human MKK polypeptide. A
substantially pure human MKK can be obtained, for
example, by extraction from a natural source; by
25 expression of a recombinant nucleic acid encoding a human
MKK polypeptide, or by chemically synthesizing the
protein. Purity can be measured by any appropriate
method, e.g., column chromatography, polyacrylamide gel
electrophoresis, or HPLC analysis.

30 In one aspect, the invention features isolated and
purified polynucleotides which encode the MKKs of the
invention. In one embodiment, the polynucleotide is the
nucleotide sequence of SEQ ID NO:1. In other
embodiments, the polynucleotide is the nucleotide

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sequence of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9, respectively.

As used herein, "polynucleotide" refers to a nucleic acid sequence of deoxyribonucleotides or ribonucleotides in the form of a separate fragment or a component of a larger construct. DNA encoding portions or all of the polypeptides of the invention can be assembled from cDNA fragments or from oligonucleotides that provide a synthetic gene which can be expressed in a recombinant transcriptional unit. Polynucleotide sequences of the invention include DNA, RNA, and cDNA sequences, and can be derived from natural sources or synthetic sequences synthesized by methods known to the art.

As used herein, an "isolated" polynucleotide is a polynucleotide that is not immediately contiguous (i.e., covalently linked) with either of the coding sequences with which it is immediately contiguous (i.e., one at the 5' end and one at the 3' end) in the naturally-occurring genome of the organism from which the polynucleotide is derived. The term therefore includes, for example, a recombinant polynucleotide which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequences.

The isolated and purified polynucleotide sequences of the invention also include polynucleotide sequences that hybridize under stringent conditions to the polynucleotide sequences specified herein. The term "stringent conditions" means hybridization conditions that guarantee specificity between hybridizing polynucleotide sequences, such as those described herein,

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or more stringent conditions. One skilled in the art can select posthybridization washing conditions, including temperature and salt concentrations, which reduce the number of nonspecific hybridizations such that only
5 highly complementary sequences are identified (Sambrook et al. (1989) in Molecular Cloning, 2d ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

The isolated and purified polynucleotide sequences of the invention also include sequences complementary to
10 the polynucleotide encoding MKK (antisense sequences). Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub (1990) *Scientific American* 262:40). The invention includes all antisense polynucleotides
15 capable of inhibiting production of MKK polypeptides. In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and
20 introduced into a target MKK-producing cell. The use of antisense methods to inhibit the translation of genes is known in the art, and is described, e.g., in Marcus-Sakura *Anal. Biochem.*, 172:289 (1988).

In addition, ribozyme nucleotide sequences for MKK
25 are included in the invention. Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences encoding these RNAs, molecules can
30 be engineered to recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech (1988) *J. Amer. Med. Assn.* 260:3030). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

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There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff (1988) Nature 334:585) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while

5 "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type

10 ribozymes for inactivating a specific mRNA species, and 18-base recognition sequences are preferable to shorter recognition sequences.

The MKK polypeptides can also be used to produce antibodies that are immunoreactive or bind epitopes of

15 the MKK polypeptides. Accordingly, one aspect of the invention features antibodies to the MKK polypeptides of the invention. The antibodies of the invention include polyclonal antibodies which consist of pooled monoclonal antibodies with different epitopic specificities, as well

20 as distinct monoclonal antibody preparations. Monoclonal antibodies are made from antigen-containing fragments of the MKK polypeptide by methods known in the art (See, for example, Kohler et al. (1975) Nature 256:495).

The term "antibody" as used herein includes intact

25 molecules as well as fragments thereof, such as Fa, F(ab')₂, and Fv, which are capable of binding the epitopic determinant. Antibodies that bind MKK polypeptides can be prepared using intact polypeptides or fragments containing small peptides of interest as the

30 immunizing antigen. The polypeptide or peptide used to immunize an animal can be derived from translated cDNA or chemically synthesized, and can be conjugated to a carrier protein, if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum

35 albumin and thyroglobulin. The coupled peptide is then

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used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

The invention also features methods of identifying subjects at risk for MKK-mediated disorders by measuring activation of the MKK signal transduction pathway. Activation of the MKK signal transduction pathway can be determined by measuring MKK synthesis; activation of MKK isoforms; activation of MKK substrates p38 or JNK isoforms; or activation of p38 and JNK substrates such as ATF2, ATFa, CRE-BPa, and c-Jun. The term "JNK" or "JNK isoforms" includes both JNK1 and JNK2. The term "MKK substrate" as used herein include MKK substrates, as well as MKK substrate substrates, e.g., p38, JNK, ATF2, and c-Jun.

In one embodiment, activation of the MKK signal transduction pathway is determined by measuring activation of the MKK signal transduction pathway substrates p38, JNK isoforms, ATF2, or c-Jun. MKK activity is measured by the rate of substrate phosphorylation as determined by quantitation of the rate of [³²]P incorporation. The specificity of MKK substrate phosphorylation can be tested by measuring p38 and JNK activation, or by employing mutated p38 and JNK molecules that lack the sites of MKK phosphorylations. Altered phosphorylation of the substrate relative to control values indicates alteration of the MKK signal transduction pathway, and increased risk in a subject of an MKK-mediated disorder. MKK activation of p38 and JNK can be detected in a coupled assay with the MKK signal transduction substrate ATF2, or related compounds such as ATFa and CRE-BPa. Activation can also be detected with the substrate c-Jun. When ATF2 is included in the assay, it is present as an intact protein or as a fragment of the intact protein, e.g., the activation domain (residues 1-109, or a portion thereof). ATF2 is incubated with a

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test sample in which MKK activity is to be measured and
[γ -³²P]ATP, under conditions sufficient to allow the
phosphorylation of ATF2. ATF2 is then isolated and the
amount of phosphorylation quantitated. In a specific
5 embodiment, ATF2 is isolated by immunoprecipitation,
resolved by SDS-PAGE, and detected by autoradiography.

In another embodiment, activation of the MKK
signal transduction pathway is determined by measuring
the level of MKK expression in a test sample. In a
10 specific embodiment, the level of MKK expression is
measured by Western blot analysis. The proteins present
in a sample are fractionated by gel electrophoresis,
transferred to a membrane, and probed with labeled
antibodies to MKK. In another specific embodiment, the
15 level of MKK expression is measured by Northern blot
analysis. Polyadenylated [poly(A)⁺] mRNA is isolated
from a test sample. The mRNA is fractionated by
electrophoresis and transferred to a membrane. The
membrane is probed with labeled MKK cDNA. In another
20 embodiment, MKK expression is measured by quantitative
PCR applied to expressed mRNA.

The MKKs of the invention are useful to screen
reagents that modulate MKK activity. MKKs are activated
by phosphorylation. Accordingly, in one aspect, the
25 invention features methods for identifying a reagent
which modulates MKK activity, by incubating MKK with the
test reagent and measuring the effect of the test reagent
on MKK synthesis, phosphorylation, function, or activity.
In one embodiment, the test reagent is incubated with MKK
30 and [³²P]-ATP, and the rate of MKK phosphorylation
determined, as described above. In another embodiment,
the test reagent is incubated with a cell transfected
with an MKK polynucleotide expression vector, and the
effect of the test reagent on MKK transcription is
35 measured by Northern blot analysis, as described above.

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In a further embodiment, the effect of the test reagent on MKK synthesis is measured by Western blot analysis using an antibody to MKK. In still another embodiment, the effect of a reagent on MKK activity is measured by incubating MKK with the test reagent, [³²]P-ATP, and a substrate in the MKK signal transduction pathway, including one or more of p38, JNK, and ATF2. The rate of substrate phosphorylation is determined as described above.

10 The term "modulation of MKK activity" includes inhibitory or stimulatory effects. The invention is particularly useful for screening reagents that inhibit MKK activity. Such reagents are useful for the treatment or prevention of MKK-mediated disorders, for example, inflammation and oxidative damage.

 The invention further features a method of treating a MKK-mediated disorder by administering to a subject in need thereof an effective dose of a therapeutic reagent that inhibits the activity of MKK.

20 By the term "MKK-mediated disorder" is meant a pathological condition resulting, at least in part, from excessive activation of an MKK signal transduction pathway. The MKK signal transduction pathways are activated by several factors, including inflammation and stress. MKK-mediated disorders include, for example, ischemic heart disease, burns due to heat or radiation (UV, X-ray, γ , β , etc.), kidney failure, liver damage due to oxidative stress or alcohol, respiratory distress syndrome, septic shock, rheumatoid arthritis, autoimmune disorders, and other types of inflammatory diseases.

30 As used herein, the term "therapeutic reagent" means any compound or molecule that achieves the desired effect on an MKK-mediated disorder when administered to a subject in need thereof.

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MKK-mediated disorders further include proliferative disorders, particularly disorders that are stress-related. Examples of stress-related MKK-mediated proliferative disorders are psoriasis, acquired immune deficiency syndrome, malignancies of various tissues of the body, including malignancies of the skin, bone marrow, lung, liver, breast, gastrointestinal system, and genito-urinary tract. Preferably, therapeutic reagents inhibit the activity or expression of MKK inhibit cell growth or cause apoptosis.

A therapeutic reagent that "inhibits MKK activity" interferes with a MKK-mediated signal transduction pathway. For example, a therapeutic reagent can alter the protein kinase activity of MKK, decrease the level of MKK transcription or translation, e.g., an antisense polynucleotide able to bind MKK mRNA, or suppress MKK phosphorylation of p38, JNK, or ATF2, thus disrupting the MKK-mediated signal transduction pathway. Examples of such reagents include antibodies that bind specifically to MKK polypeptides, and fragments of MKK polypeptides that competitively inhibit MKK polypeptide activity.

A therapeutic reagent that "enhances MKK activity" supplements a MKK-mediated signal transduction pathway. Examples of such reagents include the MKK polypeptides themselves, which can be administered in instances where the MKK-mediated disorder is caused by underexpression of the MKK polypeptide. In addition, portions of DNA encoding an MKK polypeptide can be introduced into cells that underexpress an MKK polypeptide.

A "therapeutically effective amount" is an amount of a reagent sufficient to decrease or prevent the symptoms associated with the MKK-mediated disorder.

Therapeutic reagents for treatment of MKK-mediated disorders identified by the method of the invention are administered to a subject in a number of ways known to

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the art, including parenterally by injection, infusion, sustained-release injection or implant, intravenously, intraperitoneally, intramuscularly, subcutaneously, or transdermally. Epidermal disorders and disorders of the
5 epithelial tissues are treated by topical application of the reagent. The reagent is mixed with other compounds to improve stability and efficiency of delivery (e.g., liposomes, preservatives, or dimethyl sulfoxide (DMSO)). Polynucleotide sequences, including antisense sequences,
10 can be therapeutically administered by techniques known to the art resulting in introduction into the cells of a subject suffering from the MKK-mediated disorder. These methods include the use of viral vectors (e.g., retrovirus, adenovirus, vaccinia virus, or herpes virus),
15 colloid dispersions, and liposomes.

The materials of the invention are ideally suited for the preparation of a kit for the detection of the level or activity of MKK. Accordingly, the invention features a kit comprising an antibody that binds MKK, or
20 a nucleic acid probe that hybridizes to a MKK polynucleotide, and suitable buffers. The probe or monoclonal antibody can be labeled to detect binding to a MKK polynucleotide or protein. In a preferred embodiment, the kit features a labeled antibody to MKK.

25 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein
30 can be used in the practice or testing of the present invention, the preferred methods and materials are described below. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

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Other features and advantages of the invention will be apparent from the detailed description, and from the claims.

Detailed Description

5 The drawings will first be described.

Drawings

Fig. 1 is a comparison of the amino acid sequences of MKK3 (SEQ ID NO:2), MKK4- α (SEQ ID NO:6), the human MAP kinase kinases MEK1 (SEQ ID NO:11) and MEK2 (SEQ ID
10 NO:12), and the yeast HOG1 MAP kinase kinase PBS2 (SEQ ID NO:13). MKK3 and MKK4 sequences were compared with the PILE-UP program (version 7.2; Wisconsin Genetics Computer Group). The protein sequences are presented in single letter code [A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G,
15 Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp, and Y, Tyr]. The PBS2 sequence is truncated at both the NH₂- (<) and COOH- (>) termini. Gaps introduced into the sequences to optimize the alignment are illustrated by a
20 dash. Identical residues are indicated by a period. The sites of activating phosphorylation in MEK are indicated by asterisks.

Fig. 2 is a dendrogram showing the relation between members of the human and yeast MAP kinase
25 kinases. The dendrogram was created by the unweighted pair-group method with the use of arithmetic averages (PILE-UP program). The human (hu) MAP kinase kinases MEK1, MEK2, MKK3, and MKK4; the *Saccharomyces cerevisiae* (sc) MAP kinase kinases PBS2, MKK1, and STE7; and the
30 *Saccharomyces pombe* (sp) MAP kinase kinases WIS1 and BYR1 are presented.

Fig. 3 is a schematic representation of the ERK, p38, and JNK signal transduction pathways. MEK1 and MEK2 are activators of the ERK subgroup of MAP kinase. MKK3

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and MKK4 are activators of the p38 MAP kinase. MKK4 is identified as an activator of both the p38 and JNK subgroups of MAP kinase.

Fig. 4 is a representation of the nucleic acid (SEQ ID NO:1) and amino acid sequences (SEQ ID NO:2) for MKK3.

Fig. 5 is a representation of the nucleic acid (SEQ ID NO:3) and amino acid sequences (SEQ ID NO:4) for MKK6.

Fig. 6 is a representation of the nucleic acid (SEQ ID NO:5) and amino acid sequences (SEQ ID NO:6) for MKK4 α .

Fig. 7 is a representation of the nucleic acid (SEQ ID NO:7) and amino acid sequences (SEQ ID NO:8) for MKK4 β .

Fig. 8 is a representation of the nucleic acid (SEQ ID NO:9) and amino acid sequences (SEQ ID NO:10) for MKK4 γ .

Human Mitogen-Activated Protein Kinase Kinases

The human MAP kinase kinases MKK3 and MKK4 (MKK3/4), and MKK6 described herein mediate the transduction of specific signals from the cell surface to the nucleus along specific pathways. These signal transduction pathways are initiated by factors such as cytokines, UV radiation, osmotic shock, and oxidative stress. Activation of MKK3/4 results in activation of the MAP kinases p38 (MKK3/4) and JNK (MKK4). p38 and JNK in turn activate a group of related transcription factors such as ATF2, ATF α , and CRE-BP α . These transcription factors in turn activate expression of specific genes. For example, ATF2 is known to activate expression of human T cell leukemia virus 1 (Wagner and Green (1993) Science 262:395), transforming growth factor- β 2 (Kim et al. (1992) supra), interferon- β (Du et al. (1993) Cell

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74:887), and E-selectin (DeLuca et al. (1994) J. Biol. Chem. 269:19193). In addition, ATF2 is implicated in the function of a T cell-specific enhancer (Georgopoulos et al. (1992) Mol. Cell. Biol. 12:747).

5 The isolation of human MKKs is described in Example 1 and in Dérijard et al. (1995) Science 267:682-685. Distinctive regions of the yeast PBS2 sequence were used to design polymerase chain reaction (PCR) primers. Amplification of human brain mRNA with these primers
10 resulted in the formation of specific products which were cloned into a plasmid vector and sequenced. Two different complementary DNAs (cDNAs) that encoded human protein kinases were identified: one encoding a 36 kD protein (MKK3), and one encoding a 44 kD protein (MKK4).
15 MKK4 includes 3 isoforms that vary slightly at the NH₂-terminal, identified as α , β , and γ . The amino acid sequences of MKK3 (SEQ ID NO:2), MKK4- α (SEQ ID NO:6), MKK4- β (SEQ ID NO:8), and MKK4- γ (SEQ ID NO:10) are shown in Fig. 1. The nucleic acid and amino acid sequences of
20 MKK3 (Fig. 5), MKK6 (Fig. 6), MKK4 α (Fig. 7), MKK4 β (Fig. 8), and MKK4 γ (Fig. 9) are also provided. MKK6 was isolated from a human skeletal muscle library by cross-hybridization with MKK3. Except for differences at the N-terminus, MKK6 is homologous to MKK3. Other human MKK3
25 and MKK4 isoforms that exist can be identified by the method described in Example 1.

 The expression of these human MKK isoforms was examined by Northern (RNA) blot analysis of mRNA isolated from eight adult human tissues (Example 2). Both protein
30 kinases were found to be widely expressed in human tissues, with the highest expression seen in skeletal muscle tissue.

 The substrate specificity of MKK3 was investigated in an *in vitro* phosphorylation assay with recombinant
35 epitope-tagged MAP kinases (JNK1, p38, and ERK2) as

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substrates (Example 3). MKK3 and MKK6 phosphorylated p38, but did not phosphorylate JNK1 or ERK2.

Phosphoaminoacid analysis of p38 demonstrated the presence of a phosphothreonine and phosphotyrosine.

- 5 Mutational analysis of p38 demonstrated that replacement of phosphorylation sites Thr¹⁸⁰ and Tyr¹⁸² with Ala and Phe, respectively, blocked p38 phosphorylation. These results establish that MKK3 functions *in vitro* as a p38 MAP kinase kinase. The substrate specificity of MKK6 is
10 similar to that of MKK3, but the specific activity of MKK6 is approximately 300-fold greater than that of MKK3.

Studies of the *in vitro* substrate specificity of MKK4 are described in Example 4. MKK4 incubated with [γ -³²P]ATP, and JNK1, p38, or ERK2 was found to phosphorylate
15 both p38 and JNK1. MKK4 activation of JNK and p38 was also studied by incubating MKK4 with wild-type or mutated JNK1 or p38. The p38 substrate ATF2 was included in each assay. MKK4 was found to exhibit less autophosphorylation than MKK3. MKK4 was also found to be
20 a substrate for activated MAP kinase. Unlike MKK3 and MKK6, MKK4 was also found to activate JNK1. MKK4 incubated with wild-type JNK1, but not mutated JNK1, resulted in increased phosphorylation of ATF2. These results establish that MKK4 is a p38 MAP kinase kinase
25 that also phosphorylates the JNK subgroup of MAP kinases.

In vivo activation of p38 by UV-stimulated MKK3 is described in Example 5. Cells expressing MKK3 were exposed in the presence or absence of UV radiation. MKK3 was isolated by immunoprecipitation and used for protein
30 kinase assays with the substrates p38 or JNK. ATF2 was included in some assays as a substrate for p38 and JNK. MKK3 from non-activated cultured COS cells caused a small amount of phosphorylation of p38 MAP kinase, resulting from basal activity of MKK3. MKK3 from UV-irradiated
35 cells caused increased phosphorylation of p38 MAP kinase,

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but not of JNK1. An increase in p38 activity was also detected in assays in which ATF2 was included as a substrate. These results establish that MKK3 is activated by UV radiation.

5 The effect of expression of MKK3 and MKK4 on p38 activity was examined in COS-1 cells (Example 6). Cells were transfected with a vector encoding p38 and a MEK1, MKK3, or MKK4. Some of the cells were also exposed to EGF or UV radiation. p38 was isolated by
10 immunoprecipitation and assayed for activity with [γ -³²P]ATP and ATF2. The expression of the ERK activator MEK1 did not alter p38 phosphorylation of ATF2. In contrast, expression of MKK3 or MKK4 caused increased activity of p38 MAP kinase. The activation of p38 caused
15 by MKK3 and MKK4 was similar to that observed in UV-irradiated cells, and was much greater than that detected in EGF-treated cells. These *in vitro* results provide evidence that MKK3 and MKK4 activate p38 *in vivo*.

 A series of experiments was conducted to examine
20 the potential regulation of ATF2 by JNK1. These experiments are described in Gupta et al. (1995) Science 267:389-393. The effect of UV radiation on ATF2 phosphorylation was investigated in COS-1 cells transfected with and without epitope-tagged JNK1 (Example
25 7). Cells were exposed to UV radiation, and JNK1 and JNK2 visualized by in-gel protein kinase assay with the substrate ATF2. JNK1 and JNK2 were detected in transfected and non-transfected cells exposed to UV radiation; however, JNK1 levels were higher in the
30 transfected cells. These results demonstrate that ATF2 is a substrate for the JNK1 and JNK2 protein kinases, and that these protein kinases are activated in cells exposed to UV light.

 The site of JNK1 phosphorylation of ATF2 was
35 examined by deletion analysis (Example 8). Progressive

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NH₂-terminal domain deletion GST-ATF2 fusion proteins were generated, and phosphorylation by JNK1 isolated from UV-irradiated cells was examined. The results showed that JNK1 requires the presence of ATF2 residues 1-60 for phosphorylation of the NH₂-terminal domain of ATF2.

The ATF2 residues required for binding of JNK1 were similarly examined. JNK1 was incubated with immobilized ATF2, unbound JNK1 was removed by extensive washing, and bound JNK1 was detected by incubation with [γ-³²P]ATP. Results indicate that residues 20 to 60 of ATF2 are required for binding and phosphorylation by JNK1. A similar binding interaction between ATF2 and the 55 kD JNK2 protein kinase has also been observed.

Phosphorylation by JNK1 was shown to reduce the electrophoretic mobility of ATF2 (Example 9). Phosphoamino acid analysis of the full-length ATF2 molecule (residues 1-505) demonstrated that JNK phosphorylated both Thr and Ser residues. The major sites of Thr and Ser phosphorylation were located in the NH₂ and COOH terminal domains, respectively. The NH₂-terminal sites of phosphorylation were identified as Thr⁶⁹ and Thr⁷¹ by phosphopeptide mapping and mutational analysis. These sites of Thr phosphorylation are located in a region of ATF2 that is distinct from the sub-domain required for JNK binding (residues 20 to 60).

The reduced electrophoretic mobility seen with phosphorylation of ATF2 was investigated further (Example 10). JNK1 was activated in CHO cells expressing JNK1 by treatment with UV radiation, pro-inflammatory cytokine interleukin-1 (IL-1), or serum. A decreased electrophoretic mobility of JNK1-activated ATF2 was observed in cells treated with UV radiation and IL-1. Smaller effects were seen after treatment of cells with serum. These results indicate that ATF2 is an *in vivo* substrate for JNK1.

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The effect of UV radiation on the properties of wild-type (Thr^{69,71}) and phosphorylation-defective (Ala^{69,71}) ATF2 molecules was investigated (Example 11). Exposure to UV caused a decrease in the electrophoretic mobility of both endogenous and over-expressed wild-type ATF2. This change in electrophoretic mobility was associated with increased ATF2 phosphorylation. Both the electrophoretic mobility shift and increased phosphorylation were blocked by the replacement of Thr⁶⁹ and Thr⁷¹ with Ala in ATF2. This mutation also blocked the phosphorylation of ATF2 on Thr residues *in vivo*.

Transcriptional activities of fusion proteins consisting of the GAL4 DNA binding domain and wild-type or mutant ATF2 were examined (Example 12). Point mutations at Thr⁶⁹ and/or Thr⁷¹ of ATF2 significantly decreased the transcriptional activity of ATF2 relative to the wild-type molecule, indicating the physiological relevance of phosphorylation at these sites for activity.

The binding of JNK1 to the NH₂-terminal activation domain of ATF2 (described in Example 8) suggested that a catalytically inactive JNK1 molecule could function as a dominant inhibitor of the wild-type JNK1 molecule. This hypothesis was investigated by examining the effect of a catalytically inactive JNK1 molecule on ATF2 function (Example 13). A catalytically-inactive JNK1 mutant was constructed by replacing the sites of activating Thr¹⁸³ and Tyr¹⁸⁵ phosphorylation with Ala and Phe, respectively (Ala¹⁸³, Phe¹⁸⁵, termed "dominant-negative"). Expression of wild-type JNK1 caused a small increase in serum-stimulated ATF2 transcriptional activity. In contrast, dominant-negative JNK1 inhibited both control and serum-stimulated ATF2 activity. This inhibitory effect results from the non-productive binding of the JNK1 mutant to the ATF2 activation domain, effectively blocking ATF2 phosphorylation.

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The tumor suppressor gene product Rb binds to ATF2 and increases ATF2-stimulated gene expression (Kim et al. (1992) Nature 358:331). Similarly, the adenovirus oncoprotein E1A associates with the DNA binding domain of ATF2 and increases ATF2-stimulated gene expression by a mechanism that requires the NH₂-terminal activation domain of ATF2 (Liu and Green (1994) Nature 368:520). ATF2 transcriptional activity was investigated with the luciferase reporter gene system in control, Rb-treated, and E1A-treated cells expressing wild-type or mutant ATF2 molecules (Example 14). Rb and E1A were found to increase ATF2-stimulated gene expression of both wild-type and mutant ATF2. However, mutant ATF2 caused a lower level of reporter gene expression than did wild-type ATF2. Together, these results indicate a requirement for ATF2 phosphorylation (on Thr⁶⁹ and Thr⁷¹) plus either Rb or E1A for maximal transcriptional activity. Thus, Rb and E1A act in concert with ATF2 phosphorylation to control transcriptional activity.

A series of experiments were conducted to examine the action of p38 activation and to establish the relationship of the p38 MAP kinase pathway to the ERK and JNK signal transduction pathways (Raingeaud et al. (1995) J. Biol. Chem. 270:7420). Initially, the substrate specificity of p38 was investigated by incubating p38 with proteins that have been demonstrated to be substrates for the ERK and/or JNK groups of MAP kinases (Example 15). We examined the phosphorylation of MBP (Erickson et al. (1990) J. Biol. Chem. 265:19728), EGF-R (Northwood et al. (1991) J. Biol. Chem. 266:15266), cytoplasmic phospholipase A₂ (cPLA₂) (Lin et al. (1993) Cell 72:269), c-Myc (Alvarez et al. (1991) J. Biol. Chem. 266:15277), IκB, c-Jun, and wild-type (Thr^{69,71}) or mutated (Ala^{69,71}) ATF2. p38 phosphorylated MBP and EGF-R, and to a lesser extent IκB, but not the other ERK

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substrates, demonstrating that the substrate specificity of p38 differs from both the ERK and JNK groups of MAP kinases. Wild-type ATF2, but not mutated ATF2 (Ala^{69,71}), was found to be an excellent p38 substrate.

- 5 The phosphorylation of ATF2 by p38 was associated with an electrophoretic mobility shift of ATF2 during polyacrylamide gel electrophoresis. We tested the hypothesis that p38 phosphorylates ATF2 at the same sites as JNK1 by replacing Thr⁶⁹ and Thr⁷¹ with Ala (Ala^{69,71}).
- 10 It was found that p38 did not phosphorylate mutated ATF2, which demonstrates that p38 phosphorylates ATF2 within the NH₂-terminal activation domain on Thr⁶⁹ and Thr⁷¹.

A comparison of the binding of JNK and p38 to ATF2 was conducted by incubating extracts of cells expressing
15 JNK1 or p38 with epitope alone (GST) or GST-ATF2 (residues 1-109 containing the activation domain) (Example 16). Bound protein kinases were detected by Western blot analysis. The results demonstrate that both p38 and JNK bind to the ATF2 activation domain.

- 20 EGF and phorbol ester are potent activators of the ERK signal transduction pathway (Egan and Weinberg (1993) Nature 365:781), causing maximal activation of the ERK sub-group of MAP kinases. These treatments, however, cause only a small increase in JNK protein kinase
25 activity (Dérijard et al. (1994) supra; Hibi et al. (1993) supra). The effects of EGF or phorbol esters, as well UV radiation, osmotic shock, interleukin-1, tumor necrosis factor, and LPS, on p38 activity were all tested (Example 17). Significantly, EGF and phorbol ester
30 caused only a modest increase in p38 protein kinase activity, whereas environmental stress (UV radiation and osmotic shock) caused a marked increase in the activity of both p38 and JNK. Both p38 and JNK were activated in cells treated with pro-inflammatory cytokines (TNF and
35 IL-1) or endotoxic LPS. Together, these results indicate

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that p38, like JNK, is activated by a stress-induced signal transduction pathway.

ERKs and JNKs are activated by dual phosphorylation within the motifs Thr-Glu-Tyr and Thr-Pro-Tyr, respectively. In contrast, p38 contains the related sequence Thr-Gly-Tyr. To test whether this motif is relevant to the activation of p38, the effect of the replacement of Thr-Gly-Tyr with Ala-Gly-Phe was examined (Example 18). The effect of UV radiation on cells expressing wild-type (Thr¹⁸⁰, Tyr¹⁸²) or mutant p38 (Ala¹⁸⁰, Phe¹⁸²) was studied. Western blot analysis using an anti-phosphotyrosine antibody demonstrated that exposure to UV radiation caused an increase in the Tyr phosphorylation of p38. The increased Tyr phosphorylation was confirmed by phosphoaminoacid analysis of p38 isolated from [γ -³²P]phosphate-labeled cells. This analysis also demonstrated that UV radiation caused increased Thr phosphorylation of p38. Significantly, the increased phosphorylation on Thr¹⁸⁰ and Tyr¹⁸² was blocked by the Ala¹⁸⁰/Phe¹⁸² mutation. This result demonstrates that UV radiation causes increased activation of p38 by dual phosphorylation.

It has recently been demonstrated that ERK activity is regulated by the mitogen-induced dual specificity phosphatases MKP1 and PAC1 (Ward et al. (1994) Nature 367:651). The activation of p38 by dual phosphorylation (Example 18) raises the possibility that p38 may also be regulated by dual specificity phosphatases. We examined the effect of MKP1 and PAC1 on p38 MAP kinase activation (Example 19). Cells expressing human MKP1 and PAC1 were treated with and without UV radiation, and p38 activity measured. The expression of PAC1 or MKP1 was found to inhibit p38 activity. The inhibitory effect of MKP1 was greater than PAC1. In contrast, cells transfected with a catalytically inactive

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mutant phosphatase (mutant PAC1 Cys²⁵⁷/Ser) did not inhibit p38 MAP kinase. These results demonstrate that p38 can be regulated by dual specificity phosphatases PAC1 and MKP1.

5 The sub-cellular distribution of p38 MAP kinase was examined by indirect immunofluorescence microscopy (Example 20). Epitope-tagged p38 MAP kinase was detected using the M2 monoclonal antibody. Specific staining of cells transfected with epitope-tagged p38 MAP kinase was
10 observed at the cell surface, in the cytoplasm, and in the nucleus. Marked changes in cell surface and nuclear p38 MAP kinase were not observed following UV irradiation, but an increase in the localization of cytoplasmic p38 MAP kinase to the perinuclear region was
15 detected.

A series of experiments were conducted to study the activation of JNK by hyper-osmotic media (Example 21). These experiments were reported by Galcheva-Gargova et al. (1994) Science 265:806. CHO cells expressing
20 epitope-tagged JNK1 were incubated with 0 - 1000 mM sorbitol, and JNK1 activity measured in an immune complex kinase assay with the substrate c-Jun. Increased JNK1 activity was observed in cells incubated 1 hour with 100 mM sorbitol. Increased JNK1 activity was observed within
25 5 minutes of exposure to 300 mM sorbitol. Maximal activity was observed 15 to 30 minutes after osmotic shock with a progressive decline in JNK1 activity at later times. The activation of JNK by osmotic shock was studied in cells expressing wild-type (Thr¹⁸³, Tyr¹⁸⁵) or
30 mutated (Ala¹⁸³, Phe¹⁸⁵) JNK1. JNK1 activity was measured after incubation for 15 minutes with or without 300 mM sorbitol. Cells expressing wild-type JNK1 showed increased JNK1 activity, while cells expressing mutated JNK1 did not. These results demonstrate that the JNK

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signal transduction pathway is activated in cultured mammalian cells exposed to hyper-osmotic media.

The results of the above-described experiments are illustrated in Fig. 3, which diagrams the ERK, p38, and JNK MAP kinase signal transduction pathways. ERKs are potentially activated by treatment of cells with EGF or phorbol esters. In contrast, p38 is only slightly activated under these conditions (Example 15). However, UV radiation, osmotic stress, and inflammatory cytokines cause a marked increase in p38 activity. This difference in the pattern of activation of ERK and p38 suggests that these MAP kinases are regulated by different signal transduction pathways. The molecular basis for the separate identity of these signal transduction pathways is established by the demonstration that the MAP kinase kinases that activate ERK (MEK1 and MEK2) and p38 (MKK3, MKK6, and MKK4) are distinct.

MKK isoforms are useful for screening reagents which modulate MKK activity. Described in the Use section following the examples are methods for identifying reagents capable of inhibiting or activating MKK activity.

The discovery of human MKK isoforms and MKK-mediated signal transduction pathways is clinically significant for the treatment of MKK-mediated disorders. One use of the MKK isoforms is in a method for screening reagents able to inhibit or prevent the activation of the MKK-MAP kinase- ATF2 pathways.

The following examples are meant to illustrate, not limit, the invention.

Example 1. MKK Protein Kinases

The primary sequences of MKK3 and MKK4 were deduced from the sequence of cDNA clones isolated from a human fetal brain library.

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- The primers TTYTAYGGNGCNTTTYTTYATHGA (SEQ ID NO:14) and ATBCTYTCNGGNGCCATKTA (SEQ ID NO:15) were designed based on the sequence of PBS2 (Brewster et al. (1993) Science 259:1760; Maeda et al. (1994) Nature 369:242).
- 5 The primers were used in a PCR reaction with human brain mRNA as template. Two sequences that encoded fragments of PBS2-related protein kinases were identified. Full-length human cDNA clones were isolated by screening of a human fetal brain library (Dérillard et al. (1994) supra).
- 10 The cDNA clones were examined by sequencing with an Applied Biosystems model 373A machine. The largest clones obtained for MKK3 (2030 base pairs (bp)) and MKK4 (3576 bp) contained the entire coding region of these protein kinases.
- 15 The primary structures of MKK3 (SEQ ID NO:2) and MKK4 α (SEQ ID NO:6) are shown in Fig. 1. An in-frame termination codon is located in the 5' untranslated region of the MKK3 cDNA, but not in the 5' region of the MKK4 cDNA. The MKK4 protein sequence presented starts at
- 20 the second in-frame initiation codon.
- These sequences were compared to those of the human MAP kinase kinases MEK1 (SEQ ID NO:11) and MEK2 (SEQ ID NO:12) (Zheng and Guan (1993) J. Biol. Chem 268:11435) and of the yeast MAP kinase kinase PBS2 (SEQ
- 25 ID NO:13) (Boguslawski and Polazzi (1987) Proc. Natl. Acad. Sci. USA 84:5848) (Fig. 1). The identity and similarity of the kinases with human MKK3 (between subdomains I and XI) were calculated with the BESTFIT program (version 7.2; Wisconsin Genetics Computer Group)
- 30 (percent of identity to percent of similarity): MEK1, 41%/63%; MEK2, 41%/62%; MKK4 α , 52%/73%; and PBS2, 40%/59%). The identity and similarity of the kinases with human MKK4 α were calculated to be as follows (percent of identity to percent of similarity): MEK1,
- 35 44%/63%; MEK2, 45%/61%; MKK3, 52%/73%; and PBS2, 44%/58%.

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The cDNA sequences of MKK3 and MKK4 γ have been deposited in GenBank with accession numbers L36719 and L36870, respectively. The MKK4 γ cDNA sequence contains both the cDNA sequences of MKK4 α and MKK4 β , which are
5 generated *in vivo* from alternate splicing sites. One of ordinary skill in the art can readily determine the amino acid sequences of MKK3 and MKK4 isoforms from the deposited cDNA sequences.

Human MKK6 cDNA clones were isolated from a
10 skeletal muscle library by screening with an MKK3 probe at low stringency. Mammalian MKK6 expression vectors were constructed by sub-cloning the MKK6 cDNA in the *HindIII* and *XbaI* sites of pCDNA3 (Invitrogen Inc.). The sequences of all plasmids were confirmed by automated
15 sequencing with an Applied Biosystems model 373A machine.

Example 2. Expression of MKK3 and MKK4 mRNA in Adult Human Tissue

Northern blot analysis was performed with polyadenylated [poly(A)⁺] mRNA (2 μ g) isolated from human
20 heart, brain, placenta, lung, liver, muscle, kidney, and pancreas tissues. The mRNA was fractionated by denaturing agarose gel electrophoresis and was transferred to a nylon membrane. The blot was probed with the MKK3 and MKK4 cDNA labeled by random priming
25 with [α -³²P]ATP (deoxyadenosine triphosphate) (Amersham International PLC). MKK3 and MKK4 were expressed in all tissues examined; the highest expression of MKK3 and MKK4 was found in skeletal muscle tissue.

The relation between members of the human and
30 yeast MAP kinase kinase group is presented as a dendrogram (Fig. 2). MKK3/4 form a unique subgroup of human MAP kinase kinases.

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Example 3. In Vitro Phosphorylation of p38 MAP kinase by MKK3

GST-JNK1, and GST-ERK2 have been described (Dérjard et al. (1994) supra; Gupta et al. (1995) Science 267:389; Wartmann and Davis (1994) J. Biol. Chem. 269:6695). GST-p38 MAP kinase was prepared from the expression vector pGStag (Dressier et al. (1992) Biotechniques 13:866) and a PCR fragment containing the coding region of the p38 MAP kinase cDNA. GST-MKK3 and MKK4 were prepared with pGEX3X (Pharmacia-LKB Biotechnology) and PCR fragments containing the coding region of the MKK3 and MKK4 cDNAs. The GST fusion proteins were purified by affinity chromatography with the use of GSH-agarose (Smith and Johnson (1988) Gene 67:31). The expression vectors pCMV-Flag-JNK1 and pCMV-MEK1 have been described (Dérjard et al. (1994) supra; Wartmann and Davis (1994) supra). The plasmid pCMV-Flag-p38 MAP kinase was prepared with the expression vector pCMV5 (Andersson et al. (1989) J. Biol. Chem. 264:8222) and the p38 MAP kinase cDNA. The expression vectors for MKK3 and MKK4 were prepared by subcloning of the cDNAs into the polylinker of pCDNA3 (Invitrogen). The Flag epitope (Asp-Tyr-Lys-Asp-Asp-Asp-Lys (SEQ ID NO:16); Immunex, Seattle, WA) was inserted between codons 1 and 2 of the kinases by insertional overlapping PCR (Ho et al. (1989) Gene 77:51).

Protein kinase assays were performed in kinase buffer (25 mM 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid, pH 7.4, 25 mM β -glycerophosphate, 25 mM $MgCl_2$, 2 mM dithiothreitol, and 0.1 mM orthovanadate). Recombinant GST-MKK3 was incubated with $[\gamma\text{-}^{32}P]\text{ATP}$ and buffer, GST-JNK1, GST-p38 MAP kinase, or GST-ERK2. The assays were initiated by the addition of 1 μg of substrate proteins and 50 μM $[\gamma\text{-}^{32}P]\text{ATP}$ (10 Ci/mmol) in a final volume of 25 μl . The

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reactions were terminated after 30 minutes at 25°C by addition of Laemmli sample buffer. The phosphorylation of the substrate proteins was examined after SDS-polyacrylamide gel electrophoresis (SDS-PAGE) by
5 autoradiography. Phosphoaminoacid analysis was performed by partial acid hydrolysis and thin-layer chromatography (Dérijard et al. (1994) supra; Alvarez et al. (1991) J. Biol. Chem. 266:15277). Autophosphorylation of MKK3 was observed in all groups. MKK3 phosphorylated p38 MAP
10 kinase, but not JNK1 or ERK2.

A similar insertional overlapping PCR procedure was used to replace Thr¹⁸⁰ and Tyr¹⁸² of p38, with Ala and Phe, respectively. The sequence of all plasmids was confirmed by automated sequencing on an Applied
15 Biosystems model 373A machine. GST-MKK3 was incubated with [γ -³²P]ATP and buffer, wild-type GST-p38 MAP kinase (TGY), or mutated GST-p38 MAP kinase (AGF). The phosphorylated proteins were resolved by SDS-PAGE and detected by autoradiography. Only phosphorylation of
20 wild-type p38 was observed.

MKK6 was similarly tested and shown to phosphorylate p38 MAP kinase on Thr¹⁸⁰ and Tyr¹⁸², but not JNK1 or ERK2. The specific activity of MKK6 was approximately 300-fold greater than that of MKK3.

25 **Example 4. In Vitro Phosphorylation and Activation of JNK and p38 MAP Kinase by MKK4**

Protein kinase assays were conducted as described in Example 3. Recombinant GST-MKK4 was incubated with [γ -³²P]ATP and buffer, GST-JNK1, GST-p38 MAP kinase, or
30 GST-ERK2. JNK1 and p38 were phosphorylated, as was MKK4 incubated with JNK1 and p38.

GST-MKK4 was incubated with [γ -³²P]ATP and buffer, wild-type JNK1 (Thr¹⁸³, Tyr¹⁸⁵), or mutated GST-JNK1 (Ala¹⁸³, Phe¹⁸⁵). The JNK1 substrate ATF2 (Gupta et al.

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(1995) supra) was included in each incubation. ATF2 was phosphorylated in the presence of MKK4 and wild-type JNK1. The results establish that MKK4 phosphorylates and activates both p38 and JNK1.

5 Example 5. Phosphorylation and Activation of p38 MAP Kinase by UV-stimulated MKK3

Epitope-tagged MKK3 was expressed in COS-1 cells maintained in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum (5%) (Gibco-BRL).

- 10 The cells were transfected with the lipofectamine reagent according to the manufacturer's recommendations (Gibco-BRL) and treated with UV radiation or EGF as described (Dérillard et al. (1994) supra).

- The cells were exposed in the absence and presence
15 of UV-C (40 J/m²). The cells were solubilized with lysis buffer (20 mM tris, pH 7.4, 1% Triton X-100, 10% glycerol, 137 mM NaCl, 2 mM EDTA, 25 mM β -glycerophosphate, 1 mM Na orthovanadate, 1 mM phenylmethylsulfonyl fluoride, and leupeptin (10 μ g/ml))
20 and centrifuged at 100,000 x g for 15 minutes at 4°C. MKK3 was isolated by immunoprecipitation. The epitope-tagged protein kinases were incubated for 1 hour at 4°C with the M2 antibody to the Flag epitope (IBI-Kodak) bound to protein G-Sepharose (Pharmacia-LKB
25 Biotechnology). The immunoprecipitates were washed twice with lysis buffer and twice with kinase buffer.

- Protein kinase assays were conducted with the substrate GST-p38 MAP kinase or JNK1. ATF2 was included in some assays. Basal levels of MKK3 phosphorylation of
30 p38 MAP kinase were observed. UV-irradiation resulted in increased phosphorylation of p38 MAP kinase, but not of JNK1. The increased p38 MAP kinase activity resulted in increased phosphorylation of ATF2.

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Example 6. Activation of p38 MAP Kinase in Cells
Expressing MKK3 and MKK4

COS-1 cells were transfected with epitope-tagged p38 MAP kinase, together with an empty expression vector
5 or an expression vector encoding MEK1, MKK3, or MKK4 α . Some of the cultures were exposed to UV radiation (40 J/m²) or treated with 10 nM EGF. p38 MAP kinase was isolated by immunoprecipitation with M2 monoclonal antibody, and the protein kinase activity was measured in
10 the immunocomplex with [γ -³²P]ATP and ATF2 as substrates. The product of the phosphorylation reaction was visualized after SDS-PAGE by autoradiography. ATF2 was not phosphorylated in the control MEK1, or EGF-treated groups, but was phosphorylated in the MKK3, MKK4, and UV-
15 irradiated groups. MKK3 and MKK4 phosphorylation of ATF2 was similar to that seen with p38 MAP kinase isolated from UV-irradiated cells.

Example 7. Phosphorylation of ATF2 by JNK1 and JNK2

COS-1 cells were maintained in Dulbecco's modified
20 Eagle's medium supplemented with bovine serum albumin (5%) (Gibco-BRL). Metabolic labeling with [³²]P was performed by incubation of cells for 3 hours in phosphate-free modified Eagle's medium (Flow Laboratories Inc.) supplemented with [³²P]orthophosphate (2 mCi/ml)
25 (Dupont-NEN). COS-1 cells were transfected without (Mock) and with epitope-tagged JNK1 (JNK1). Plasmid expression vectors encoding the JNK1 cDNA have previously been described (Dérillard et al. (1994) Cell 76:1025). Plasmid DNA was transfected into COS-1 cells by the
30 lipofectamine method (Gibco-BRL). After 48 hours of incubation, some cultures were exposed to 40 J/m² UV radiation and incubated for 1 hour at 37°C.

Cells were lysed in 20 mM Tris, pH 7.5, 25 mM β -glycerophosphate, 10% glycerol, 1% Triton X-100, 0.5%

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(w/v) deoxycholate, 0.1% (w/v) SDS, 0.137 M NaCl, 2 mM pyr phosphate, 1 mM orthovanadate, 2 mM EDTA, 10 µg/ml leupeptin, 1 mM PMSF. Soluble extracts were prepared by centrifugation in a microfuge for 20 minutes at 4°C.

- 5 JNK1 immunoprecipitates were also prepared by reaction with a rabbit antiserum prepared with recombinant JNK1 as an antigen.

In-gel protein kinase assays were performed with cell lysates and JNK1 immunoprecipitates after SDS-PAGE
10 by renaturation of protein kinases, polymerization of the substrate (GST-ATF2, residues 1-505) in the gel, and incubation with [γ -³²P]ATP (Dériard et al. (1994) supra). The incorporation of [³²P]phosphate was visualized by autoradiography and quantitated with a Phosphorimager and
15 ImageQuant soft-ware (Molecular Dynamics Inc., Sunnyvale, CA). The cell lysates demonstrate the presence of 46 kD and 55 kD protein kinases that phosphorylate ATF2 in extracts prepared from UV-irradiated cells. The 46 kD and 55 kD protein kinases were identified as JNK1 and
20 JNK2, respectively.

Example 8. Binding of JNK1 to ATF2 and Phosphorylation of the NH₂-Terminal Activation Domain

The site of JNK1 phosphorylation of ATF2 was investigated by generation of progressive NH₂-terminal
25 domain deletions of ATF2. Plasmid expression vectors encoding ATF2 (pECE-ATF2) (Liu and Green (1994) and (1990)), have been described. Bacterial expression vectors for GST-ATF2 fusion proteins were constructed by sub-cloning ATF2 cDNA fragments from a polymerase chain
30 reaction (PCR) into pGEX-3X (Pharmacia-LKB Biotechnology Inc.). The sequence of all constructed plasmids was confirmed by automated sequencing with an Applied Biosystems model 373A machine. The GST-ATF2 proteins were purified as described (Smith and Johnson (1988) Gene

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67:31), resolved by SDS-PAGE and stained with Coomassie blue. GST-ATF2 fusion proteins contained residues 1-505, 1-349, 350-505, 1-109, 20-109, 40-109, and 60-109.

The phosphorylation of GST-ATF2 fusion proteins by
5 JNK1 isolated from UV-irradiated cells was examined in an immunocomplex kinase assay. Immunecomplex kinase assays were performed with Flag epitope-tagged JNK1 and the monoclonal antibody M2 (IBI-Kodak) as described by Dérijard et al. (1994) supra. Immunecomplex protein
10 kinase assays were also performed with a rabbit antiserum prepared with recombinant JNK1 as an antigen. The cells were solubilized with 20 mM Tris, pH 7.5, 10% glycerol, 1% Triton X-100, 0.137 M NaCl, 25 mM β -glycerophosphate, 2 mM EDTA, 1 mM orthovanadate, 2 mM pyrophosphate, 10
15 μ g/ml leupeptin, and 1 mM PMSF. JNK1 was immunoprecipitated with protein G-Sepharose bound to a rabbit polyclonal antibody to JNK or the M2 monoclonal antibody to the Flag epitope. The beads were washed three times with lysis buffer and once with kinase buffer
20 (20 mM Hepes, pH 7.6, 20 mM $MgCl_2$, 25 mM β -glycerophosphate, 100 μ M Na orthovanadate, 2 mM dithiothreitol). The kinase assays were performed at 25°C for 10 minutes with 1 μ g of substrate, 20 μ M adenosine triphosphate and 10 μ Ci of [γ - 32 P]ATP in 30 μ l of
25 kinase buffer. The reactions were terminated with Laemmli sample buffer and the products were resolved by SDS-PAGE (10% gel). JNK1 phosphorylates GST-ATF2 fusion proteins containing residues 1-505, 1-349, 1-109, 20-109, and 40-109, but not 60-109. These results indicate that
30 the presence of ATF2 residues 1-60 are required for phosphorylation by JNK.

The binding of immobilized GST-ATF2 fusion proteins was examined in a solid-phase kinase assay as described by Hibi et al. (1993) Genes Dev. 7:2135. JNK1
35 from UV-irradiated cells was incubated with GST-ATF2

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fusion proteins bound to GSH-agarose. The agarose beads were washed extensively to remove the unbound JNK1. Phosphorylation of the GST-ATF2 fusion proteins by the bound JNK1 protein kinase was examined by addition of [γ -³²P]ATP. JNK1 bound GST-ATF2 fusion proteins containing residues 1-505, 1-349, 1-109, 20-109, and 40-109, indicating that the presence of residues 20-60 were required for binding of JNK1 to ATF2.

Example 9. Phosphorylation of the NH₂-terminal
10 Activation Domain of ATF2 on Thr⁶⁹ and Thr⁷¹
by JNK1

The effect of UV radiation on the properties of wild-type (Thr^{69,71}) and phosphorylation-defective (Ala^{69,71}) ATF2 molecules was examined. Mock-transfected and JNK1-transfected COS cells were treated without and with 40 J/m² UV radiation. The epitope-tagged JNK1 was isolated by immunoprecipitation with the M2 monoclonal antibody. The phosphorylation of GST-ATF2 (residues 1 to 109) was examined in an immunocomplex kinase assay as described above. The GST-ATF2 was resolved from other proteins by SDS-PAGE and stained with Coomassie blue. The phosphorylation of GST-ATF2 was detected by autoradiography. JNK1-transfected cells, but not mock-transfected cells, phosphorylated ATF2. JNK1 phosphorylation of ATF2 was greater in cells exposed to UV radiation. Phosphorylation of ATF2 by JNK1 was associated with a decreased electrophoretic mobility.

In a separate experiment, GST fusion proteins containing full-length ATF2 (residues 1 to 505), an NH₂-terminal fragment (residues 1 to 109), and a COOH-terminal fragment (residues 95 to 505) were phosphorylated with JNK1 and the sites of phosphorylation analyzed by phosphoamino acid analysis. The methods used for phosphopeptide mapping and phosphoamino acid analysis

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have been described (Alvarez et al. (1991) J. Biol. Chem. 266:15277). The horizontal dimension of the peptide maps was electrophoresis and the vertical dimension was chromatography. The NH₂-terminal sites of phosphorylation were identified as Thr⁶⁹ and Thr⁷¹ by phosphopeptide mapping and mutational analysis. Site-directed mutagenesis was performed as described above, replacing Thr⁶⁹ and Thr⁷¹ with Ala. Phosphorylation of mutated ATF2 was not observed.

10 Example 10. Reduced Electrophoretic Mobility of JNK-Activated ATF2

CHO cells were maintained in Ham's F12 medium supplemented with 5% bovine serum albumin (Gibco-BRL). Cells were labeled and transfected with JNK1 as described above. CHO cells were treated with UV-C (40 J/m²), IL-1 α (10 ng/ml) (Genzyme), or fetal bovine serum (20%) (Gibco-BRL). The cells were incubated for 30 minutes at 37°C prior to harvesting. The electrophoretic mobility of ATF2 after SDS-PAGE was examined by protein immuno-blot analysis. A shift in ATF2 electrophoretic mobility was observed in cells treated with UV, IL-1, and serum. These results indicate that JNK1 activation is associated with an electrophoretic mobility shift of ATF2, further suggesting that ATF2 is an *in vivo* substrate for JNK1.

25 Example 11. Increased ATF2 Phosphorylation After Activation of JNK

COS-1 cells were transfected without (control) and with an ATF2 expression vector (ATF2), as described above (Hai et al. (1989) *supra*). The effect of exposure of the cells to 40 J/m² UV-C was examined. After irradiation, the cells were incubated for 0 or 30 minutes (control) or 0, 15, 30, and 45 minutes (ATF2) at 37°C and then collected. The electrophoretic mobility of ATF2 during

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SDS-PAGE was examined by protein immuno-blot analysis as described above. The two electrophoretic mobility forms of ATF2 were observed in ATF2-transfected cells, but not in control cells.

- 5 The phosphorylation state of wild-type (Thr^{69,71}) ATF2 and mutated (Ala^{69,71}) ATF2 was examined in cells labeled with [³²]P, treated without and with 40 J/m² UV-C, and then incubated at 37°C for 30 minutes (Hai et al. (1989) supra). The ATF2 proteins were isolated by
- 10 immunoprecipitation and analyzed by SDS-PAGE and autoradiography. The phosphorylated ATF2 proteins were examined by phosphoamino acid analysis as described above. Both forms of ATF2 contained phosphoserine, but only wild-type ATF2 contained phosphothreonine.
- 15 Tryptic phosphopeptide mapping was used to compare ATF2 phosphorylated *in vitro* by JNK1 with ATF2 phosphorylated in COS-1 cells. A map was also prepared with a sample composed of equal amounts of *in vivo* and *in vitro* phosphorylated ATF2 (Mix). Mutation of ATF2 at
- 20 Thr⁶⁹ and Thr⁷¹ resulted in the loss of two tryptic phosphopeptides in maps of ATF2 isolated from UV-irradiated cells. These phosphopeptides correspond to mono- and bis-phosphorylated peptides containing Thr⁶⁹ and Thr⁷¹. Both of these phosphopeptides were found in maps
- 25 of ATF2 phosphorylated by JNK1 *in vitro*.

Example 12. Inhibition of ATF2-Stimulated Gene Expression by Mutation of the Phosphorylation Sites Thr⁶⁹ and Thr⁷¹

- A fusion protein consisting of ATF2 and the GAL4
- 30 DNA binding domain was expressed in CHO cells as described above. The activity of the GAL4-ATF2 fusion protein was measured in co-transfection assays with the reporter plasmid pG5E1bLuc (Seth et al. (1992) J. Biol. Chem. 267:24796. The reporter plasmid contains five GAL4

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sites cloned upstream of a minimal promoter element and the firefly luciferase gene. Transfection efficiency was monitored with a control plasmid that expresses β -galactosidase (pCH110; Pharmacia-LKB Biotechnology). The
 5 luciferase and β -galactosidase activity detected in cell extracts was measured as the mean activity ratio of three experiments (Gupta et al. (1993) Proc. Natl. Acad. Sci. USA 90:3216). The results, shown in Table 1, demonstrate the importance of phosphorylation at Thr⁶⁹ and Thr⁷¹ for
 10 transcriptional activity.

TABLE 1. INHIBITION OF ATF-2 STIMULATED GENE EXPRESSION BY MUTATION OF THE PHOSPHORYLATION SITES THR^{69,71}

PROTEIN	LUCIFERASE ACTIVITY (Light Units/OD)
GAL4	45
15 GAL4-ATF2 (wild type)	320,000
GAL4-ATF2 (Ala ⁶⁹)	24,000
GAL4-ATF2 (Ala ⁷¹)	22,000
GAL4-ATF2 (Ala ^{69,71})	29,000
GAL4-ATF2 (Glu ⁶⁹)	27,000

20 Example 13. Effect of Dominant-Negative JNK1 Mutant on ATF2 Function

The luciferase reporter plasmid system was used to determine the effect of point mutations at the ATF2 phosphorylation sites Thr⁶⁹ and Thr⁷¹ in serum-treated CHO
 25 cells transfected with wild-type (Thr¹⁸³, Tyr¹⁸⁵) or mutant (Ala¹⁸³, Phe¹⁸⁵) JNK1. Control experiments were done with mock-transfected cells. The CHO cells were serum-starved for 18 hours and then incubated without or with serum for 4 hours. Expression of wild-type ATF2
 30 caused a small increase in serum-stimulated ATF2 transcriptional activity. In contrast, mutant JNK1 inhibited both control and serum-stimulated ATF2 activity.

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Example 14. Effect of Tumor Suppressor Gene Product Rb and Adenovirus Oncoprotein E1A on ATF2-Stimulated Gene Expression

The effect of expression of the Rb tumor suppressor gene product and adenovirus oncoprotein E1A on ATF2 transcriptional activity were investigated with a luciferase reporter plasmid and GAL4-ATF2 (residues 1-505), as described above. Cells were transfected with wild-type (Thr^{69,71}) or mutated (Ala^{69,71}) ATF2. No effect of Rb or E1A on luciferase activity was detected in the absence of GAL4-ATF2. Rb and E1A were found to increase ATF2-stimulated gene expression of both wild-type and mutated ATF2. However, mutated ATF2 caused a lower level of reporter gene expression than did wild-type ATF2. These results indicate a requirement for ATF2 phosphorylation (on Thr⁶⁹ and Thr⁷¹) plus either Rb or E1A for maximal transcriptional activity.

Example 15. Substrate Specificity of p38 MAP Kinase

Substrate phosphorylation by p38 MAP kinase was examined by incubation of bacterially-expressed p38 MAP kinase with IκB, cMyc, EGF-R, cytoplasmic phospholipase A₂ (cPLA₂), c-Jun, and mutated ATF2 (Thr^{69,71}) and ATP[γ-³²P] (Raingeaud et al. (1995) J. Biol. Chem 270:7420. GST-IκB was provided by Dr D. Baltimore (Massachusetts Institute of Technology). GST-cMyc (Alvarez et al. (1991) J. Biol. Chem. 266:15277), GST-EGF-R (residues 647-688) (Koland et al. (1990) Biochem. Biophys. Res. Commun. 166:90), and GST-c-Jun (Dérijard et al. (1994) supra) have been described. The phosphorylation reaction was terminated after 30 minutes by addition of Laemmli sample buffer. The phosphorylated proteins were resolved by SDS-PAGE and detected by autoradiography. The rate phosphorylation of the substrate proteins was quantitated by PhosphorImager (Molecular Dynamics Inc.) analysis.

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The relative phosphorylation of ATF2, MBP, EGF-R, and IxB was 1.0, 0.23, 0.04, and 0.001, respectively.

Example 16. Binding of p38 MAP Kinase to ATF2

Cell extracts expressing epitope-tagged JNK1 and
5 p38 MAP kinase were incubated with a GST fusion protein containing the activation domain of ATF2 (residues 1-109) immobilized on GSH agarose. The supernatant was removed and the agarose was washed extensively. Western blot analysis of the supernatant and agarose-bound fractions
10 was conducted as follows: proteins were fractionated by SDS-PAGE, electrophoretically transferred to an Immobilon-P membrane, and probed with monoclonal antibodies to phosphotyrosine (PY20) and the Flag epitope (M2). Immunocomplexes were detected using enhanced
15 chemiluminescence (Amersham International PLC). Control experiments were performed using immobilized GST.

Example 17. p38 MAP Kinase and JNK1 Activation by Pro-Inflammatory Cytokines and Environmental Stress

20 The effect of phorbol ester, EGF, UV radiation, osmotic stress, IL-1, tumor necrosis factor (TNF), and LPS on p38 MAP kinase and JNK1 activity were measured in immunocomplex protein kinase assays using ATP[γ -³²P] and ATF2 as substrates. TNF α and IL-1 α were from Genzyme
25 Corp. Lipopolysaccharide (LPS) was isolated from lyophilized *Salmonella minnesota* Re595 bacteria as described (Mathison et al. (1988) J. Clin. Invest. 81:1925). Phorbol myristate acetate was from Sigma. EGF was purified from mouse salivary glands (Davis (1988) J.
30 Biol. Chem. 263:9462). Kinase assays were performed using immunoprecipitates of p38 and JNK. The immunocomplexes were washed twice with kinase buffer (described above), and the assays initiated by the

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addition of 1 μ g of ATF2 and 50 μ M [γ - 32 P]ATP (10 Ci/mmol) in a final volume of 25 μ l. The reactions were terminated after 30 minutes at 30°C by addition of Laemmli sample buffer. The phosphorylation of ATF2 was examined after SDS-PAGE by autoradiography, and the rate of ATF2 phosphorylation quantitated by PhosphorImager analysis.

The results are shown in Table 2. Exposure of HeLa cells to 10 nM phorbol myristate acetate very weakly activated p38 and JNK1. Similarly, treatment with 10 nM EGF only weakly activated p38 and JNK1. By contrast, treatment with 40 J/m² UV-C, 300 mM sorbitol, 10 ng/ml interleukin-1, and 10 ng/ml TNF α strongly activated p38 and JNK1 activity. The effect of LPS on the activity of p38 was examined using CHO cells that express human CD14. Exposure of CHO cells to 10 ng/ml LPS only slightly activated p38 and JNK1 activity.

TABLE 2. p38 AND JNK1 ACTIVATION BY PRO-INFLAMMATORY CYTOKINES AND ENVIRONMENTAL STRESS.

	Relative Protein Kinase Activity	
	JNK	p38
Control	1.0	1.0
Epidermal Growth Factor (10 nM)	1.9	2.1
Phorbol Ester (10 nM)	2.3	2.9
Lipopolysaccharide (10 ng/ml)	3.6	3.7
Osmotic Shock (300 mM sorbitol)	18.1	4.2
Tumor Necrosis Factor (10 ng/ml)	19.3	10.3
Interleukin-1 (10 ng/ml)	8.9	6.2
UV (40 J/m ²)	7.4	17.1

Example 18. p38 MAP Kinase Activation by Dual Phosphorylation on Tyr and Thr

COS-1 cells expressing wild-type (Thr¹⁸⁰, Tyr¹⁸²) or mutated (Ala¹⁸⁰, Phe¹⁸²) p38 MAP kinase were treated

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without and with UV-C (40 J/m^2). The cells were harvested 30 minutes following exposure with or without UV radiation. Control experiments were performed using mock-transfected cells. The level of expression of epitope-tagged p38 MAP kinase and the state of Tyr phosphorylation of p38 MAP kinase was examined by Western blot analysis using the M2 monoclonal antibody and the phosphotyrosine monoclonal antibody PY20. Immune complexes were detected by enhanced chemiluminescence.

Wild-type and mutant p38 were expressed at similar levels. Western blot analysis showed that UV radiation caused an increase in the Tyr phosphorylation of p38. The increased Tyr phosphorylation was confirmed by phosphoamino acid analysis of p38 isolated from [^{32}P]phosphate-labeled cells. The results also showed that UV radiation increased Thr phosphorylation of p38. The increased phosphorylation on Tyr and Thr was blocked by mutated p38. Wild-type and mutated p38 were isolated from the COS-1 cells by immunoprecipitation. Protein kinase activity was measured in the immune complex using [γ - ^{32}P]ATP and GST-ATF2 as substrates. The phosphorylated GST-ATF2 was detected after SDS-PAGE by autoradiography. UV radiation resulted in a marked increase in the activity of wild-type p38, while the mutant p38 was found to be catalytically inactive. These results show that p38 is activated by dual phosphorylation within the Thr-Gly-Tyr motif.

Example 19. MAP Kinase Phosphatase Inhibits p38 MAP Kinase Activation

The cells were treated without and with 40 J/m^2 UV-C. Control experiments were performed using mock-transfected cells (control) and cells transfected with the catalytically inactive mutated phosphatase mPAC1 (Cys²⁵⁷/Ser) and human MKP1. The activity of p38 MAP

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kinase was measured with an immunocomplex protein kinase assay employing [γ - 32 P]ATP and GST-ATF2 as substrates. The expression of PAC1 or MKP1 was found to inhibit p38 phosphorylation, demonstrating that p38 can be regulated
5 by the dual specificity phosphatases PAC1 and MKP1.

Example 20. Subcellular Distribution of p38 MAP Kinase

Epitope-tagged p38 MAP kinase was expressed in COS cells. The cells were treated without or with 40 J/m² UV radiation and then incubated for 60 minutes at
10 37°C. The p38 MAP kinase was detected by indirect immunofluorescence using the M2 monoclonal antibody. The images were acquired by digital imaging microscopy and processed for image restoration.

Immunocytochemistry. Coverslips (22mm x 22mm No. 1; Gold Seal Cover Glass; Becton-Dickinson) were pre-
15 treated by boiling in 0.1 N HCl for 10 minutes, rinsed in distilled water, autoclaved and coated with 0.01% poly-L-lysine (Sigma; St. Louis MO). The coverslips were placed at the bottom of 35 mm multiwell tissue culture plates
20 (Becton Dickinson, UK). Transfected COS-1 cells were plated directly on the coverslips and allowed to adhere overnight in Dulbecco's modified Eagle's medium supplemented with 5% fetal calf serum (Gibco-BRL). 24
hours post-transfection, the cells were rinsed once and
25 incubated at 37°C for 30 minutes in 25 mM Hepes, pH 7.4, 137 mM NaCl, 6 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 10 mM glucose. The cells were rinsed once with phosphate-buffered saline and the coverslips removed from the tissue culture wells. Cells were fixed in fresh 4%
30 paraformaldehyde in phosphate-buffered saline for 15 minutes at 22°C. The cells were permeabilized with 0.25% Triton X-100 in phosphate-buffered saline for 5 minutes and washed three times in DWB solution (150 mM NaCl, 15 mM Na citrate, pH 7.0, 2% horse serum, 1% (w/v) bovine

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serum albumin, 0.05% Triton X-100) for 5 minutes. The primary antibody (M2 anti-FLAG monoclonal antibody, Eastman-Kodak Co., New Haven, CT) was diluted 1:250 in DWB and applied to the cells in a humidified environment at 22°C for 1 hour. The cells were again washed three times as above and fluorescein isothiocyanate-conjugated goat anti-mouse Ig secondary antibody (Kirkegaard & Perry Laboratories Inc. Gaithersburg, MD) was applied at a 1:250 dilution for 1 hour at 22°C in a humidified environment. The cells were then washed three times in DWB and then mounted onto slides with Gel-Mount (Biomedica Corp. Foster City, CA) for immunofluorescence analysis. Control experiments were performed to assess the specificity of the observed immunofluorescence. No fluorescence was detected when the transfected cells were stained in the absence of the primary M2 monoclonal antibody, or mock-transfected cells.

Digital Imaging Microscopy and Image Restoration

Digital images of the fluorescence distribution in single cells were obtained using a Nikon 60x Planapo objective (numerical aperture = 1.4) on a Zeiss IM-35 microscope equipped for epifluorescence as previously described (Carrington et al. (1990) in: Non-invasive Techniques in Cell Biology (Fosbett & Grinstein, eds.), Wiley-Liss, NY; pp. 53-72; Fay et al. (1989) J. Microsci. 153:133-149). Images of various focal planes were obtained with a computer controlled focus mechanism and a thermoelectrically cooled charged-coupled device camera (model 220; Photometrics Ltd., Tucson, AZ). The exposure of the sample to the excitation source was determined by a computer-controlled shutter and wavelength selector system (MVI, Avon, MA). The charge-coupled device camera and microscope functions were controlled by a microcomputer, and the data acquired from the camera were transferred to a Silicon Graphics model 4D/GTX

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workstation (Mountainview, CA) for image processing. Images were corrected for non-uniformities in sensitivity and for the dark current of the charge coupled device detector. The calibration of the microscopy blurring was
5 determined by measuring the instrument's point spread function as a series of optical sections at $0.125\mu\text{m}$ intervals of a $0.3\mu\text{m}$ diameter fluorescently labeled latex bead (Molecular Probes Inc.). The image restoration algorithm used is based upon the theory of
10 ill-posed problems and obtains quantitative dye density values within the cell that are substantially more accurate than those in an un-processed image (Carrington et al. (1990) supra; Fay et al. (1989) supra). After image processing, individual optical sections of cells
15 were inspected and analyzed using computer graphics software on a Silicon Graphics workstation. p38 MAP kinase was observed at the cell surface, in the cytoplasm, and in the nucleus. After irradiation, an increased localization of cytoplasmic p38 to the
20 perinuclear region was detected.

Example 21. Activation of the MKK Signal Transduction Pathway by Osmotic Shock

CHO cells were co-transfected with the plasmid pCMV-Flag-Jnk1 and pRSV-Neo (Dérillard et al. (1994)
25 supra). A stable cell line expressing epitope-tagged Jnk1 (Flag; Immunex Corp.) was isolated by selection with Geneticin (Gibco-BRL). The cells were incubated with 0, 100, 150, 300, 600, or 1000 mM sorbitol for 1 hour at 37°C . The cells were collected in lysis buffer (20 mM
30 Tris, pH 7.4, 1% Triton X-100, 2 mM EDTA, 137 mM NaCl, 25 mM β -glycerophosphate, 1 mM orthovanadate, 2 mM pyrophosphate, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 $\mu\text{g/ml}$ leupeptin) and a soluble extract was obtained by centrifugation at 100,000 g for 30 minutes at

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4°C. The epitope-tagged JNK1 was isolated by immunoprecipitation with the monoclonal antibody M2 (Immunex Corp.). The immunoprecipitates were washed extensively with lysis buffer. Immune complex kinase assays were done in 25 µl of 25 mM Hepes, pH 7.4, 25 mM MgCl₂, 25 mM β-glycerophosphate, 2 mM dithiothreitol, 100 µM orthovanadate, and 50 µM ATP [γ-³²P] (10 Ci/mmol) with 2.5 µg of bacterially expressed c-Jun (residues 1-79) fused to glutathione-S-transferase (GST) as a substrate. The phosphorylation of c-Jun was examined after SDS-PAGE by autoradiography and PhosphorImager (Molecular Dynamics Inc.) analysis. JNK1 activation was observed at all concentrations of sorbitol exposure.

The time course of JNK1 protein kinase activation was measured in cells incubated in medium supplemented with 300 mM sorbitol as described above. Increased JNK1 activity was observed within 5 minutes of exposure to sorbitol, with maximum activity occurring after 15-30 minutes.

Mutation of JNK1 at the phosphorylation sites Thr¹⁸³ and Tyr¹⁸⁵ blocked the activation of JNK1 protein kinase activity by osmotic shock. CHO cells were transfected with vector, wild-type JNK1 (Thr¹⁸³, Tyr¹⁸⁵), and mutated JNK1 (Ala¹⁸³, Phe¹⁸⁵). The cells were incubated in medium supplemented without or with 300 mM sorbitol for 15 minutes before measurement of JNK1 protein kinase activity as described above. JNK1 activation was seen in the wild-type but not mutated JNK1.

30 Use

The MKK polypeptides and polynucleotides of the invention are useful for identifying reagents which modulate the MKK signal transduction pathways. Reagents that modulate an MKK signal transduction pathway can be

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identified by their effect on MKK synthesis, MKK phosphorylation, or MKK activity. For example, the effect of a reagent on MKK activity can be measured by the *in vitro* kinase assays described above. MKK is
5 incubated without (control) and with a test reagent under conditions sufficient to allow the components to react, then the effect of the test reagent on kinase activity is subsequently measured. Reagents that inhibit an MKK signal transduction pathway can be used in the treatment
10 of MKK-mediated disorders. Reagents that stimulate an MKK signal transduction pathway can be used in a number of ways, including induction of programmed cell death (apoptosis) in tissues. For example, the elimination of UV damaged cells can be used to prevent cancer.

15 Generally, for identification of a reagent that inhibits the MKK signal transduction pathway, the kinase assay is tested with a range of reagent concentrations, e.g., 1.0 nM to 100 mM, a MKK substrate, and a radioactive marker such as [γ - 32 P]ATP. Appropriate
20 substrate molecules include p38, JNK1, JNK2, or ATF2. The incorporation of [32]P into the substrate is determined, and the results obtained with the test reagent compared to control values. Of particular interest are reagents that result in inhibition of [32]P
25 of about 80% or more.

Assays that test the effect of a reagent on MKK synthesis can also be used to identify compounds that inhibit MKK signal transduction pathways. The effect of the test reagent on MKK expression is measured by, for
30 example, Western blot analysis with an antibody specific for MKK. Antibody binding is visualized by autoradiography or chemiluminescence, and is quantitated. The effect of the test reagent on MKK mRNA expression can be examined, for example, by Northern blot analysis using
35 a polynucleotide probe or by polymerase chain reaction.

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Reagents found to inhibit MKK signal transduction pathways can be used as therapeutic agents for the treatment of MKK-mediated disorders. Such reagents are also useful in drug design for elucidation of the
5 specific molecular features needed to inhibit MKK signal transduction pathways.

In addition, the invention provides a method for the treatment of MKK-mediated stress-related and inflammatory disorders. The method includes
10 administration of an effective amount of a therapeutic reagent that inhibits MKK function. Suitable reagents inhibit either MKK activity or expression. The concentration of the reagent to be administered is determined based on a number of factors, including the
15 appropriate dosage, the route of administration, and the specific condition being treated. The appropriate dose of a reagent is determined by methods known to those skilled in the art including routine experimentation to optimize the dosage as necessary for the individual
20 patient and specific MKK-mediated disorder being treated. Specific therapeutically effective amounts appropriate for administration are readily determined by one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences. 18th ed., Gennaro, ed., Mack
25 Publishing Company, Easton, PA, 1990).

The invention provides methods for both acute and prophylactic treatment of stress-related and inflammatory disorders. For example, it is envisioned that ischemic heart disease will be treated during episodes of ischemia
30 and oxidative stress following reperfusion. In addition, a patient at risk for ischemia can be treated prior to ischemic episodes.

In another example, a therapeutic agent which inhibits MKK function or activity is administered to
35 control inflammatory responses by inhibiting the

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secretion of inflammatory cytokines, including TNF and IL-1.

Stress-related proliferative disorders can also be treated by the method of the invention by administering a
5 therapeutic reagent that inhibits MKK function or activity. Such therapeutic reagents can be used alone or in combination with other therapeutic reagents, for example, with chemotherapeutic agents in the treatment of malignancies. Indeed, the control of stress-activated
10 MKK by the therapeutic reagents provided by this invention can modulate symptoms caused by other therapeutic strategies that induce stress.

The therapeutic reagents employed are compounds which inhibit MKK function or activity, including
15 polynucleotides, polypeptides, and other molecules such as antisense oligonucleotides and ribozymes, which can be made according to the invention and techniques known to the art. Polyclonal or monoclonal antibodies (including fragments or derivatives thereof) that bind epitopes of
20 MKK also can be employed as therapeutic reagents. Dominant-negative forms of MKK which effectively displace or compete with MKK for substrate binding and/or phosphorylation can be used to decrease protein kinase activity. Dominant-negative forms can be created by
25 mutations within the catalytic domain of the protein kinases, as described above.

In some cases, augmentation of MKK activity is desirable, e.g., induction of apoptosis. The methods of the invention can be used to identify reagents capable of
30 increasing MKK function or activity. Alternatively, increased activity is achieved by over-expression of MKK. When a MKK-mediated disorder is associated with underexpression of MKK, or expression of a mutant MKK polypeptide, a sense polynucleotide sequence (the DNA

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coding strand) or MKK polypeptide can be introduced into the cell.

The antibodies of the invention can be administered parenterally by injection or by gradual
5 infusion over time. The monoclonal antibodies of the invention can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

Preparations for parenteral administration of a
10 polypeptide or an antibody of the invention include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as
15 ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed
20 oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose) and the like. Preservatives and other additives can also be present, such as, for example, antimicrobials, antioxidants, chelating agents,
25 and inert gases, and the like.

Polynucleotide sequences, including antisense sequences, can be therapeutically administered by various techniques known to those skilled in the art. Such therapy would achieve its therapeutic effect by
30 introduction of the MKK polynucleotide into cells of mammals having a MKK-mediated disorder. Delivery of MKK polynucleotides can be achieved using free polynucleotide or a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially

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preferred for therapeutic delivery of nucleotide sequences is the use of targeted liposomes.

Targeting of the therapeutic reagent to specific tissues is desirable to increase the efficiency of delivery. The targeting can be achieved by passive mechanisms via the route of administration. Active targeting to specific tissues can also be employed. The use of liposomes, colloidal suspensions, and viral vectors allows targeting to specific tissues by changing the composition of the formulation containing the therapeutic reagent, for example, by including molecules that act as receptors for components of the target tissues. Examples include sugars, glycolipids, polynucleotides, or proteins. These molecules can be included with the therapeutic reagent. Alternatively, these molecules can be included by indirect methods, for example, by inclusion of a polynucleotide that encodes the molecule, or by use of packaging systems that provide targeting molecules. Those skilled in the art will know, or will ascertain with the use of the teaching provided herein, which molecules and procedures will be useful for delivery of the therapeutic reagent to specific tissues.

Other Embodiments

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Davis, Roger J.
Raingeaud, Joel
Gupta, Shashi
Derijard, Benoit
- (ii) TITLE OF INVENTION: CYTOKINE-, STRESS-, AND
ONCOPROTEIN-ACTIVATED HUMAN PROTEIN KINASE
KINASES
- (iii) NUMBER OF SEQUENCES: 16
- (iv) CORRESPONDENCE ADDRESS:
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 - (E) COUNTRY: USA
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- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
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- (viii) ATTORNEY/AGENT INFORMATION:
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 - (B) REGISTRATION NUMBER: 32,983
 - (C) REFERENCE/DOCKET NUMBER: 07917/010001
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 - (B) TELEFAX: 617/542-8906
 - (C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2030 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- 50 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GCGAGGAGCG TGGTCCCCAC CCATCCAGCC CATATGTGCA AGTGCCCTTG ACAGAGAGGC	180
TGGTCATATC CATGGTGACC ATTTATGGGC CACAACAGGT CCCCATCTGC GCAGTGAACC	240
CTGTGCTGAG CACCTTGACG ACGTGATCTT GCTTCGTCCT GCAGCACTGT GCGGGGCAGG	300
AAAATCCAAG AGGAAGAAGG ATCTACGGAT ATCCTGCATG TCCAAGCCAC CCGCACCCAA	360
CCCCACACCC CCGCGAACC TGGACTCCCG GACCTTCATC ACCATTGGAG ACAGAAACTT	420
TGAGGTGGAG GCTGATGACT TGGTGACCAT CTCAGAACTG GGCCGTGGAG CCTATGGGGT	480
GGTAGAGAAG GTGCGGCACG CCCAGAGCGG CACCATCATG GCCGTGAAGC GGATCCGGGC	540
CACCGTGAAC TCACAGGAGC AGAAGCGGCT GCTCATGGAC CTGGACATCA ACATGCGCAC	600
GGTCGACTGT TTCTACACTG TCACCTTCTA CGGGGCACTA TTCAGAGAGG GAGACGTGTG	660
GATCTGCATG GAGCTCATGG ACACATCCTT GGACAAGTTC TACCGGAAGG TGCTGGATAA	720
AAACATGACA ATTCCAGAGG ACATCCTTGG GGAGATTGCT GTGTCTATCG TGCGGGCCCT	780
GGAGCATCTG CACAGCAAGC TGTGCGTGAT CCACAGAGAT GTGAAGCCCT CCAATGTCCT	840
TATCAACAAG GAGGGCCATG TGAAGATGTG TGACTTTGGC ATCAGTGGCT ACTTGGTGGA	900
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CCCAGAGCTG AACCAGAAGG GCTACAATGT CAAGTCCGAC GTCTGGAGCC TGGGCATCAC	1020
CATGATTGAG ATGGCCATCC TGCGGTTCCC TTACGAGTCC TGGGGGACCC CGTTCCAGCA	1080
GCTGAAGCAG GTGGTGGAGG AGCGGTCCCC CCAGCTCCCA GCGACCGTT TCTCCCCGA	1140
GTTTGTGGAC TTCACTGCTC AGTGCCTGAG GAAGAACCCG GCAGAGCGTA TGAGCTACCT	1200
GGAGCTGATG GAGCACCCCT TCTTCACCTT GCACAAAACC AAGAAGACGG ACATTGCTGC	1260
CTTCGTGAAG AAGATCCTGG GAGAAGACTC ATAGGGGCTG GGCCTCGGAC CCCACTCCGG	1320
CCCTCCAGAG CCCCACAGCC CCATCTGCGG GGGCAGTGCT CACCCACACC ATAAGCTACT	1380
GCCATCCTGG CCCAGGGCAT CTGGGAGGAA CCGAGGGGCG TGCTCCCACC TGGCTCTGTG	1440
GCGAGCCATT TGTCCCAAGT GCCAAAGAAG CAGACCATTG GGGCTCCCAG CCAGGCCCTT	1500
GTGCGCCCCA CCAAGTCCTC TCCCTGCTGC TCCTAGGACC CGTCTCCAGC TGCTGAGATC	1560
CTGGACTGAG GGGGCTTGGG TGCCCCCTGT GGATGCTGCT GCCCTGCAC AGCAGGCTGC	1620
CAGTGCTTGG GTGGATGGGC CACCGCCTTG CCCAGCCTGG ATGCCATCCA AGTTGTATAT	1680
TTTTTTAATC TCTCGACTGA ATGGACTTTG CACACTTTGG CCCAGGTGG CCACACCTCT	1740
ATCCCGGCTT TGGTGCGGGG TACACAAGAG GGGATGAGTT GTGTGAATAC CCCAAGACTC	1800
CCATGAGGGA GATGCCATGA GCCGCCAAG GCCTTCCCCT GGCCTGGCA AACAGGGCCT	1860

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CTGCGGAGCA CACTGGCTCA CCCAGTCCTG CCCGCCACCG TTATCGGTGT CATTACCTT 1920
 TCGTGTTTTT TTTAATTTAT CCTCTGTTGA TTTTTCCTT TGCTTTATGG GTTTGGCTTG 1980
 TTTTCTTGC ATGGTTTGA GCTGATCGCT TCTCCCCAC CCCCTAGGGG 2030

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 318 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met	Ser	Lys	Pro	Pro	Ala	Pro	Asn	Pro	Thr	Pro	Pro	Arg	Asn	Leu	Asp	1	5	10	15
Ser	Arg	Thr	Phe	Ile	Thr	Ile	Gly	Asp	Arg	Met	Phe	Glu	Val	Glu	Ala	20	25	30	
Asp	Asp	Leu	Val	Thr	Ile	Ser	Glu	Leu	Gly	Arg	Gly	Ala	Tyr	Gly	Val	35	40	45	
Val	Glu	Lys	Val	Arg	His	Ala	Gln	Ser	Gly	Thr	Ile	Met	Ala	Val	Lys	50	55	60	
Arg	Ile	Arg	Ala	Thr	Val	Asn	Ser	Gln	Glu	Gln	Lys	Arg	Leu	Leu	Met	65	70	75	80
Asp	Leu	Asp	Ile	Asn	Met	Arg	Thr	Val	Asp	Cys	Phe	Tyr	Thr	Val	Thr	85	90	95	
Phe	Tyr	Gly	Ala	Leu	Phe	Arg	Glu	Gly	Asp	Val	Trp	Ile	Cys	Met	Glu	100	105	110	
Leu	Met	Asp	Thr	Ser	Leu	Asp	Lys	Phe	Tyr	Arg	Lys	Val	Leu	Asp	Lys	115	120	125	
Asn	Met	Thr	Ile	Pro	Glu	Asp	Ile	Leu	Gly	Glu	Ile	Ala	Val	Ser	Ile	130	135	140	
Val	Arg	Ala	Leu	Glu	His	Leu	His	Ser	Lys	Leu	Ser	Val	Ile	His	Arg	145	150	155	160
Asp	Val	Lys	Pro	Ser	Asn	Val	Leu	Ile	Asn	Lys	Glu	Gly	His	Val	Lys	165	170	175	
Met	Cys	Asp	Phe	Gly	Ile	Ser	Gly	Tyr	Leu	Val	Asp	Ser	Val	Ala	Lys	180	185	190	
Thr	Met	Asp	Ala	Gly	Cys	Lys	Pro	Tyr	Met	Ala	Pro	Glu	Arg	Ile	Asn	195	200	205	
Pro	Glu	Leu	Asn	Gln	Lys	Gly	Tyr	Asn	Val	Lys	Ser	Asp	Val	Trp	Ser	210	215	220	
Leu	Gly	Ile	Thr	Met	Ile	Glu	Met	Ala	Ile	Leu	Arg	Phe	Pro	Tyr	Glu	225	230	235	240
Ser	Trp	Gly	Thr	Pro	Phe	Gln	Gln	Leu	Lys	Gln	Val	Val	Glu	Glu	Pro	245	250	255	

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Ser Pro Gln Leu Pro Ala Asp Arg Phe Ser Pro Glu Phe Val Asp Phe
 260 265 270

Thr Ala Gln Cys Leu Arg Lys Asn Pro Ala Glu Arg Met Ser Tyr Leu
 275 280 285

Glu Leu Met Glu His Pro Phe Phe Thr Leu His Lys Thr Lys Lys Thr
 290 295 300

Asp Ile Ala Ala Phe Val Lys Lys Ile Leu Gly Glu Asp Ser
 305 310 315

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1602 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TAGCTGCAGC ACAGCCTTCC CTAACGTTGC AACTGGGGGA AAAATCACTT TCCAGTCTGT	60
TTTGCAAGGT GTGCATTTCC ATCTTGATTC CCTGAAAGTC CATCTGCTGC ATCGGTCAAG	120
AGAAACTCCA CTTGCATGAA GATTGCACGC CTGCAGCTTG CATCTTTGTT GCAAAACTAG	180
CTACAGAAGA GAAGCAAGGC AAAGTCTTTT GTGCTCCCTT CCCCCATCAA AGGAAAGGGG	240
AAAATGTCTC AGTCGAAAGG CAAGAAGCGA AACCTGGCC TTAATAATTC AAAAGAAGCA	300
TTTGAACAAC CTCAGACCAG TTCCACACCA CCTAGAGATT TAGACTCCAA GGCTTGCAAT	360
TCTATTGGAA ATCAGAACTT TGAGGTGAAG GCAGATGACC TGGAGCCTAT AATGGAAGTG	420
GGACGAGGTG CGTACGGGGT GGTGGAGAAG ATGCGGCACG TGCCCAGCGG GCAGATCATG	480
GCAGTGAAGC GGATCCGAGC CACAGTAAAT AGCCAGGAAC AGAAACGGCT ACTGATGGAT	540
TGGATATTT CCATGAGGAC GGTGGACTGT CCATTCACCTG TCACCTTTTA TGGCGCACTG	600
TTTCGGGAGG GTGATGTGTG GATCTGCATG GAGCTCATGG ATACATCACT AGATAAATTC	660
TACAAACAAG TTATTGATAA AGGCCAGACA ATTCCAGAGG ACATCTTAGG GAAAATAGCA	720
GTTTCTATTG TAAAAGCATT AGAACATTTA CATAGTAAGC TGTCTGTCAT TCACAGAGAC	780
GTCAAGCCTT CTAATGTACT CATCAATGCT CTCGGTCAAG TGAAGATGTG CGATTTTGGA	840
ATCAGTGGCT ACTTGGTGGA CTCGTGCTTCT AAAACAATTG ATGCAGGTTG CAAACCATAC	900
ATGGCCCCCTG AAAGAATAAA CCCAGAGCTC AACCGAAGG GATACAGTGT GAAGTCTGAC	960
ATTTGGAGTC TGGGCATCAC GATGATTGAG TTGGCCATCC TTCGATTTCC CTATGATTCA	1020
TGGGGAACCTC CATTTAGCA GCTCAAACAG GTGGTAGAGG AGCCATCGCC ACAACTCCCA	1080
GCAGACAAGT TCTCTGCAGA GTTTGTTGAC TTTACCTCAC AGTGCTTAAA GAAGAATTCC	1140
AAAGAACGGC CTACATACCC AGAGCTAATG CAACATCCAT TTTTACCCTT ACATGAATCC	1200
AAAGGAACAG ATGTGGCATC TTTTGTAATA CTGATTCTTG GAGACTAAAA AGCAGTGGAC	1260

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TTAATCGGTT GACCCTACTG TGGATTGGTG GGTTTCGGGG TGAAGCAAGT TCACTACAGC 1320
 ATCAATAGAA AGTCATCTTT GAGATAATTT AACCCCTGCCT CTCAGAGGGT TTTCTCTCCC 1380
 AATTTTCTTT TTAATCCCCC TCTTAAGGGG GCCTTGGAAT CTATAGTATA GAATGAACTG 1440
 TCTAGATGGA TGAATTATGA TAAAGGCTTA GGACTTCAAA AGGTGATTAA ATATTTAATG 1500
 ATGTGTCATA TGAGTCCTCA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1560
 AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AA 1602

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 334 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ser Gln Ser Lys Gly Lys Lys Arg Asn Pro Gly Leu Lys Ile Pro
 1 5 10 15
 Lys Glu Ala Phe Glu Gln Pro Gln Thr Ser Ser Thr Pro Pro Arg Asp
 20 25 30
 Leu Asp Ser Lys Ala Cys Ile Ser Ile Gly Asn Gln Asn Phe Glu Val
 35 40 45
 Lys Ala Asp Asp Leu Glu Pro Ile Met Glu Leu Gly Arg Gly Ala Tyr
 50 55 60
 Gly Val Val Glu Lys Met Arg His Val Pro Ser Gly Gln Ile Met Ala
 65 70 75 80
 Val Lys Arg Ile Arg Ala Thr Val Asn Ser Gln Glu Gln Lys Arg Leu
 85 90 95
 Leu Met Asp Leu Asp Ile Ser Met Arg Thr Val Asp Cys Pro Phe Thr
 100 105 110
 Val Thr Phe Tyr Gly Ala Leu Phe Arg Glu Gly Asp Val Trp Ile Cys
 115 120 125
 Met Glu Leu Met Asp Thr Ser Leu Asp Lys Phe Tyr Lys Gln Val Ile
 130 135 140
 Asp Lys Gly Gln Thr Ile Pro Glu Asp Ile Leu Gly Lys Ile Ala Val
 145 150 155 160
 Ser Ile Val Lys Ala Leu Glu His Leu His Ser Lys Leu Ser Val Ile
 165 170 175
 His Arg Asp Val Lys Pro Ser Asn Val Leu Ile Asn Ala Leu Gly Gln
 180 185 190
 Val Lys Met Cys Asp Phe Gly Ile Ser Gly Tyr Leu Val Asp Ser Val
 195 200 205
 Ala Lys Thr Ile Asp Ala Gly Cys Lys Pro Tyr Met Ala Pro Glu Arg
 210 215 220

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Ile Asn Pro Glu Leu Asn Gln Lys Gly Tyr Ser Val Lys Ser Asp Ile
 225 230 235 240

Trp Ser Leu Gly Ile Thr Met Ile Glu Leu Ala Ile Leu Arg Phe Pro
 245 250 255

Tyr Asp Ser Trp Gly Thr Pro Phe Gln Gln Leu Lys Gln Val Val Glu
 260 265 270

Glu Pro Ser Pro Gln Leu Pro Ala Asp Lys Phe Ser Ala Glu Phe Val
 275 280 285

Asp Phe Thr Ser Gln Cys Leu Lys Lys Asn Ser Lys Glu Arg Pro Thr
 290 295 300

Tyr Pro Glu Leu Met Gln His Pro Phe Phe Thr Leu His Glu Ser Lys
 305 310 315 320

Gly Thr Asp Val Ala Ser Phe Val Lys Leu Ile Leu Gly Asp
 325 330

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3497 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTAGGGTCCC CGGCGCCAGG CCACCCGGCC GTCAGCAGCA TGCAGGGTAA ACGCAAAGCA 60

CTGAAGTTGA ATTTTGCAAA TCCACCTTTC AAATCTACAG CAAGGTTTAC TCTGAATCCC 120

AATCCTACAG GAGTTCAAAA CCCACACATA GAGAGACTGA GAACACACAG CATTGAGTCA 180

TCAGGAAAAC TGAAGATCTC CCCTGAACAA CACTGGGATT TCACTGCAGA GGACTTGAAA 240

GACCTTGGAG AAATTGGACG AGGAGCTTAT GGTTCGTCA ACAAAATGGT CCACAAACCA 300

AGTGGGCAAA TAATGGCAGT TAAAAGAATT CGGTCAACAG TGGATGAAAA AGAACAAAAA 360

CAACTCTTA TGGATTGGA TGTAGTAATG CGGAGTAGTG ATTGCCATA CATTGTTCAG 420

TTTTATGGTG CACTCTTCAG AGAGGGTGAC TGTGGATCT GTATGGAACAT CATGTCTACC 480

TCGTTTGATA AGTTTACAA ATATGTATAT AGTGTATTAG ATGATGTTAT TCCAGAAGAA 540

ATTTTAGGCA AAATCACTTT AGCAACTGTG AAAGCACTAA ACCACTTAAA AGAAAACCTG 600

AAAATTATTC ACAGAGATAT CAAACCTTCC AATATTCTTC TGGACAGAAG TGGAAATATT 660

AAGCTCTGTG ACTTCGGCAT CAGTGGACAG CTTGTGGACT CTATTGCCAA GACAAGAGAT 720

GCTGGCTGTA GGCCATACAT GGCACCTGAA AGAATAGACC CAAGCGCATC ACGACAAGGA 780

TATGATGTCC GCTCTGATGT CTGGAGTTTG GGGATCACAT TGTATGAGTT GGCCACAGGC 840

CGATTTCCTT ATCCAAAGTG GAATAGTGT TTTGATCAAC TAACACAAGT CGTGAAAGGA 900

GATCCTCCGC AGCTGAGTAA TTCTGAGGAA AGGGAATTCT CCCCGAGTTT CATCAACTTT 960

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GTCAACTTGT GCCTTACGAA GGATGAATCC AAAAGGCCAA AGTATAAAGA GCTTCTGAAA	1020
CATCCCTTTA TTTTGATGTA TGAAGAACGT GCCGTTGAGG TCGCATGCTA TGTTTGTA	1080
ATCCTGGATC AAATGCCAGC TACTCCCAGC TCTCCCATGT ATGTCGATTG ATATCGTGCT	1140
ACATCAGACT CTAGAAAAAA GGGCTGAGAG GAAGCAAGAC GTAAAGAATT TTCATCCCGT	1200
ATCACAGTGT TTTTATTGCT CGCCCAGACA CCATGTGCAA TAAGATTGGT GTTCGTTTCC	1260
ATCATGTCTG TATACTCCTG TCACCTAGAA CGTGCATCCT TGTAATACCT GATTGATCAC	1320
ACAGTGTTAG TGCTGGTCAG AGAGACCTCA TCCTGCTCTT TTGTGATGAA CATATTCATG	1380
AAATGTGGAA GTCAGTACGA TCAAGTTGTT GACTGTGATT AGATCACATC TTAAATTCAT	1440
TTCTAGACTC AAAACCTGGA GATGCAGCTA CTGGAATGGT GTTTTGTGAG ACTTCCAAAT	1500
CCTGGAAGGA CACAGTGATG AATGTACTAT ATCTGAACAT AGAAACTCGG GCTTGAGTGA	1560
GAAGAGCTTG CACAGCCAAC GAGACACATT GCCTTCTGGA GCTGGGAGAC AAAGGAGGAA	1620
TTTACTTTCT TCACCAAGTG CAATAGATTA CTGATGTGAT ATTCTGTTGC TTTACAGTTA	1680
CAGTTGATGT TTGGGGATCG ATGTGCTCAG CCAAATTTCC TGTTTGAAAT ATCATGTAA	1740
ATTAGAATGA ATTTATCTTT ACCAAAAACC ATGTTGCGTT CAAAGAGGTG AACATTAAAA	1800
TATAGAGACA GGACAGAATG TGTTCCTTTT TCCTCTACCA GTCCTATTTT TCAATGGGAA	1860
GACTCAGGAG TCTGCCACTT GTCAAAGAAG GTGCTGATCC TAAGAAATTT TCATTCTCAG	1920
AATTCGGTGT GCTGCCAACT TGATGTTCCA CCTGCCACAA ACCACCAGGA CTGAAGAAG	1980
AAAACAGTAC AGAAGGCAAA GTTTACAGAT GTTTTAAATT CTAGTATTTT ATCTGGAACA	2040
ACTTGTAGCA GCTATATATT TCCCCTTGGT CCCAAGCCTG ATACTTTAGC CATCATAACT	2100
CACTAACAGG GAGAAGTAGC TAGTAGCAAT GTGCCTTGAT TGATTAGATA AAGATTCTA	2160
GTAGGCAGCA AAAGACCAAA TCTCAGTTGT TTGCTTCTTG CCATCACTGG TCCAGGTCTT	2220
CAGTTTCCGA ATCTCTTTCC CTTCOCCTGT GGTCTATTGT CGCTATGTGA CTTGCGCTTA	2280
ATCCAATATT TTGCCTTTTT TCTATATCAA AAAACCTTTA CAGTTAGCAG GGATGTTCC	2340
TACCGAGGAT TTTTAACCCC CAATCTCTCA TAATCGCTAG TGTTTAAAAG GCTAAGAATA	2400
GTGGGGCCCA ACCGATGTGG TAGGTGATAA AGAGGCATCT TTTCTAGAGA CACATTGGAC	2460
CAGATGAGGA TCCGAAACGG CAGCCTTTAC GTTCATCACC TGCTAGAACC TCTCGTAGTC	2520
CATCACCATT TCTTGCCATT GGAATTCTAC TGGAAAAAA TACAAAAAGC AAAACAAAAC	2580
CCTCAGCACT GTTACAAGAG GCCATTTAAG TATCTTGTC TTCTTCACTT ACCCATTAGC	2640
CAGGTTCTCA TTAGGTTTTG CTGGGCCTC CCTGGCACTG AACCTTAGGC TTTGTATGAC	2700
AGTGAAGCAG CACTGTGAGT GGTTCAGCA CACTGGAATA TAAACAGTC ATGGCCTGAG	2760
ATGCAGGTGA TGCCATTACA GAACCAATC GTGGCACGTA TTGCTGTGTC TCCTCTCAGA	2820
GTGACAGTCA TAAATACTGT CAAACAATAA AGGGAGAATG GTGCTGTTA AAGTCACATC	2880

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CCTGTAAATT GCAGAATTCA AAAGTGATTA TCTCTTTGAT CTACTTGCCT CATTTCCCTA 2940
 TCTTCTCCCC CACGGTATCC TAAACTTTAG ACTTCCCACT GTTCTGAAAG GAGACATTGC 3000
 TCTATGTCTG CCTTCGACCA CAGCAAGCCA TCATCCTCCA TTGCTCCCGG GGA CTCAAGA 3060
 GGAATCTGTT TCTCTGCTGT CAACTTCCCA TCTGGCTCAG CATAGGGTCA CTTTGCCATT 3120
 ATGCAAATGG AGATAAAGC AATTCTGGCT GTCCAGGAGC TAATCTGACC GTTCTATTGT 3180
 GTGGATGACC ACATAAGAAG GCAATTTTAG TGTATTAATC ATAGATTATT ATAAACTATA 3240
 AACTTAAGGG CAAGGAGTTT ATTACAATGT ATCTTTATTA AAACAAAAGG GTGTATAGTG 3300
 TTCACAACT GTGAAAATAG TGTAAGAACT GTACATTGTG AGCTCTGGTT ATTTTCTCT 3360
 TGTACCATAG AAAAATGTAT AAAAATTATC AAAAAGCTAA TGTGCAGGGA TATTGCCTTA 3420
 TTTGTCTGTA AAAAATGGAG CTCAGTAACA TAACTGCTTC TTGGAGCTTT GGAATATTTT 3480
 ATCTGTATT CTTGTTT 3497

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 363 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: not relevant
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Gln Gly Lys Arg Lys Ala Leu Lys Leu Asn Phe Ala Asn Pro Pro
 1 5 10 15
 Phe Lys Ser Thr Ala Arg Phe Thr Leu Asn Pro Asn Pro Thr Gly Val
 20 25 30
 Gln Asn Pro His Ile Glu Arg Leu Arg Thr His Ser Ile Glu Ser Ser
 35 40 45
 Gly Lys Leu Lys Ile Ser Pro Glu Gln His Trp Asp Phe Thr Ala Glu
 50 55 60
 Asp Leu Lys Asp Leu Gly Glu Ile Gly Arg Gly Ala Tyr Gly Ser Val
 65 70 75 80
 Asn Lys Met Val His Lys Pro Ser Gly Gln Ile Met Ala Val Lys Arg
 85 90 95
 Ile Arg Ser Thr Val Asp Glu Lys Glu Gln Lys Gln Leu Leu Met Asp
 100 105 110
 Leu Asp Val Val Met Arg Ser Ser Asp Cys Pro Tyr Ile Val Gln Phe
 115 120 125
 Tyr Gly Ala Leu Phe Arg Glu Gly Asp Cys Trp Ile Cys Met Glu Leu
 130 135 140
 Met Ser Thr Ser Phe Asp Lys Phe Tyr Lys Tyr Val Tyr Ser Val Leu
 145 150 155 160

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Asp Asp Val Ile Pro Glu Glu Ile Leu Gly Lys Ile Thr Leu Ala Thr
 165 170 175
 Val Lys Ala Leu Asn His Leu Lys Glu Asn Leu Lys Ile Ile His Arg
 180 185 190
 Asp Ile Lys Pro Ser Asn Ile Leu Leu Asp Arg Ser Gly Asn Ile Lys
 195 200 205
 Leu Cys Asp Phe Gly Ile Ser Gly Gln Leu Val Asp Ser Ile Ala Lys
 210 215 220
 Thr Arg Asp Ala Gly Cys Arg Pro Tyr Met Ala Pro Glu Arg Ile Asp
 225 230 235 240
 Pro Ser Ala Ser Arg Gln Gly Tyr Asp Val Arg Ser Asp Val Trp Ser
 245 250 255
 Leu Gly Ile Thr Leu Tyr Glu Leu Ala Thr Gly Arg Phe Pro Tyr Pro
 260 265 270
 Lys Trp Asn Ser Val Phe Asp Gln Leu Thr Gln Val Val Lys Gly Asp
 275 280 285
 Pro Pro Gln Leu Ser Asn Ser Glu Glu Arg Glu Phe Ser Pro Ser Phe
 290 295 300
 Ile Asn Phe Val Asn Leu Cys Leu Thr Lys Asp Glu Ser Lys Arg Pro
 305 310 315 320
 Lys Tyr Lys Glu Leu Leu Lys His Pro Phe Ile Leu Met Tyr Glu Glu
 325 330 335
 Arg Ala Val Glu Val Ala Cys Tyr Val Cys Lys Ile Leu Asp Gln Met
 340 345 350
 Pro Ala Thr Pro Ser Ser Pro Met Tyr Val Asp
 355 360

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3553 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAACAATGGC GGCTCCGAGC CCGAGCGGTG GCGGCGGCAG CGGCACCCCC GGCCCCGTAG	60
GGTCCCCGGC GCCAGGCCAC CCGGCCGTCA GCAGCATGCA GGGTAAACGC AAAGCACTGA	120
AGTTGAATTT TGCAAATCCA CCTTTCAAAT CTACAGCAAG GTTTACTCTG AATCCCAATC	180
CTACAGGAGT TCAAAACCCA CACATAGAGA GACTGAGAAC ACACAGCATT GAGTCATCAG	240
GAAAACTGAA GATCTCCCTT GAACAACACT GGGATTTCAC TGCAGAGGAC TTGAAAGACC	300
TTGGAGAAAT TGGACGAGGA GCTTATGGTT CTGTCAACAA AATGGTCCAC AAACCAAGTG	360
GGCAAATAAT GGCAGTTAAA AGAATTCGGT CAACAGTGGT TGAAAAAGAA CAAAAACAAC	420

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TTCTTATGGA	TTTGGATGTA	GTAATGCGGA	GTAGTGATTG	CCCATACATT	GTTCAGTTTT	480
ATGGTGCACT	CTTCAGAGAG	GGTGACTGTT	GGATCTGTAT	GGAACATCATG	TCTACCTCGT	540
TTGATAAGTT	TTACAAATAT	GTATATAGTG	TATTAGATGA	TGTTATTCCA	GAAGAAATTT	600
TAGGCAAAAT	CACTTTAGCA	ACTGTGAAAG	CACTAAACCA	CTTAAAGAA	AACTTGAAAA	660
TTATTCACAG	AGATATCAAA	CCTTCCAATA	TTCTTCTGGA	CAGAAGTGGA	AATATTAAAGC	720
TCTGTGACTT	CGGCATCAGT	GGACAGCTTG	TGGACTCTAT	TGCCAAGACA	AGAGATGCTG	780
GCTGTAGGCC	ATACATGGCA	CCTGAAAGAA	TAGACCCAAG	CGCATCACGA	CAAGGATATG	840
ATGTCGCTC	TGATGTCTGG	AGTTTGGGGA	TCACATTGTA	TGAGTTGGCC	ACAGGCCGAT	900
TTCCTTATCC	AAAGTGGAAT	AGTGTATTTG	ATCAACTAAC	ACAAGTCGTG	AAAGGAGATC	960
CTCCGAGCT	GAGTAATTCT	GAGGAAAGGG	AATTCTCCCC	GAGTTTCATC	AACTTTGTCA	1020
ACTTGTGCCT	TACGAAGGAT	GAATCCAAAA	GGCCAAAGTA	TAAAGAGCTT	CTGAAACATC	1080
CCTTTATTTT	GATGTATGAA	GAACGTGCCG	TTGAGGTGCG	ATGCTATGTT	TGTAAATCC	1140
TGGATCAAAT	GCCAGCTACT	CCCAGCTCTC	CCATGTATGT	CGATTGATAT	CGTGCTACAT	1200
CAGACTCTAG	AAAAAAGGGC	TGAGAGGAAG	CAAGACGTAA	AGAATTTTCA	TCCCGTATCA	1260
CAGTGTTTTT	ATTGCTCGCC	CAGACACCAT	GTGCAATAAG	ATTGGTGTTT	GTTTCCATCA	1320
TGTCTGTATA	CTCCTGTCAC	CTAGAACGTG	CATCCTTGTA	ATACCTGATT	GATCACACAG	1380
TGTTAGTGCT	GGTCAGAGAG	ACCTCATCCT	GCTCTTTTGT	GATGAACATA	TTCATGAAAT	1440
GTGGAAGTCA	GTACGATCAA	GTTGTTGACT	GTGATTAGAT	CACATCTTAA	ATTCATTCT	1500
AGACTCAAAA	CCTGGAGATG	CAGCTACTGG	AATGGTGTTT	TGTCAGACTT	CCAAATCCTG	1560
GAAGGACACA	GTGATGAATG	TACTATATCT	GAACATAGAA	ACTCGGGCTT	GAGTGAGAAG	1620
AGCTTGACAC	GCCAAACGAG	CACATTGCCT	TCTGGAGCTG	GGAGACAAAG	GAGGAATTTA	1680
CTTTCTTCAC	CAAGTGCAAT	AGATTACTGA	TGTGATATTC	TGTTGCTTTA	CAGTTACAGT	1740
TGATGTTTGG	GGATCGATGT	GCTCAGCCAA	ATTTCTGTG	TGAAATATCA	TGTTAAATTA	1800
GAATGAATTT	ATCTTTACCA	AAAACCATGT	TGCGTTCAAA	GAGGTGAACA	TTAAATATA	1860
GAGACAGGAC	AGAATGTGTT	CTTTCTCCT	CTACCAGTCC	TATTTTCAA	TGGGAAGACT	1920
CAGGAGTCTG	CCACTTGTC	AAGAAGGTGC	TGATCCTAAG	AAATTTTCAT	TCTCAGAATT	1980
CGGTGTGCTG	CCAACCTGAT	GTTCCACCTG	CCACAAACCA	CCAGGACTGA	AAGAAGAAAA	2040
CAGTACAGAA	GGCAAAGTTT	ACAGATGTTT	TTAATTCTAG	TATTTTATCT	GGAACAACCT	2100
GTAGCAGCTA	TATATTTCCC	CTTGGTCCCA	AGCCTGATAC	TTTAGCCATC	ATAACTCACT	2160
AACAGGGAGA	AGTAGCTAGT	AGCAATGTGC	CTTGATTGAT	TAGATAAAGA	TTTCTAGTAG	2220
GCAGCAAAAG	ACCAAATCTC	AGTTGTTTGC	TTCTTGCCAT	CACTGGTCCA	GGTCTTCAGT	2280
TTCCGAATCT	CTTCCCTTC	CCCTGTGGTC	TATTGTGCT	ATGTGACTTG	CGCTTAATCC	2340

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AATATTTTGC CTTTTTCTA TATCAAAAA CCTTTACAGT TAGCAGGGAT TTCCTTACC	2400
GAGGATTTTT AACCCCAAT CTCTCATAAT CGCTAGTGT TAAAGGCTA AGAATAGTGG	2460
GGCCCAACCG ATGTGGTAGG TGATAAGAG GCATCTTTTC TAGAGACACA TTGGACCAGA	2520
TGAGGATCCG AAACGGCAGC CTTTACGTT ATCACCTGCT AGAACCTCTC GTAGTCCATC	2580
ACCATTCTT GGCATTGGAA TTCTACTGGA AAAAAATACA AAAAGCAAAA CAAAACCTC	2640
AGCACTGTTA CAAGAGGCCA TTTAAGTATC TTGTGCTTCT TCACTTACCC ATTAGCCAGG	2700
TTCTCATTAG GTTTTGCTTG GGCCTCCCTG GCACTGAACC TTAGGCTTTG TATGACAGTG	2760
AAGCAGCACT GTGAGTGGTT CAAGCACACT GGAATATAAA ACAGTCATGG CCTGAGATGC	2820
AGGTGATGCC ATTACAGAAC CAAATCGTGG CACGTATTGC TGTGTCTCCT CTCAGAGTGA	2880
CAGTCATAAA TACTGTCAA CAATAAGGG AGAATGGTGC TGTTTAAAGT CACATCCCTG	2940
TAAATTGCAG AATTCAAAG TGATTATCTC TTTGATCTAC TTGCCTCATT TCCCTATCTT	3000
CTCCCCACG GTATCCTAAA CTTTAGACTT CCCACTGTTC TGAAAGGAGA CATTGCTCTA	3060
TGTCTGCCCT CGACCACAGC AAGCCATCAT CCTCCATTGC TCCCGGGGAC TCAAGAGGAA	3120
TCTGTTTCTC TGCTGTCAAC TTCCCATCTG GCTCAGCATA GGGTCACTTT GCCATTATGC	3180
AAATGGAGAT AAAAGCAATT CTGGCTGTCC AGGAGCTAAT CTGACCGTTC TATTGTGTGG	3240
ATGACCACAT AAGAAGGCAA TTTTAGTGTA TTAATCATAG ATTATTATAA ACTATAAACT	3300
TAAGGGCAAG GAGTTTATTA CAATGTATCT TTATTAAAC AAAAGGGTGT ATAGTGTTCA	3360
CAAAGTGTGA AAATAGTGTA AGAACTGTAC ATTGTGAGCT CTGGTTATTT TTCTCTTGTA	3420
CCATAGAAAA ATGTATAAAA ATTATCAAAA AGCTAATGTG CAGGGATATT GCCTTATTTG	3480
TCTGTAAAAA ATGGAGCTCA GTAACATAAC TGCTTCTTGG AGCTTTGGAA TATTTTATCC	3540
TGTATTCTTG TTT	3553

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 393 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met	Ala	Ala	Pro	Ser	Pro	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Thr	Pro	Gly
1				5				10						15	
Pro	Val	Gly	Ser	Pro	Ala	Pro	Gly	His	Pro	Ala	Val	Ser	Ser	Met	Gln
		20						25					30		
Gly	Lys	Arg	Lys	Ala	Leu	Lys	Leu	Asn	Phe	Ala	Asn	Pro	Pro	Phe	Lys
		35					40					45			
Ser	Thr	Ala	Arg	Phe	Thr	Leu	Asn	Pro	Asn	Pro	Thr	Gly	Val	Gln	Asn
	50					55					60				

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Pro His Ile Glu Arg Leu Arg Thr His Ser Ile Glu S r Ser Gly Lys
 65 70 75 80
 Leu Lys Ile Ser Pro Glu Gln His Trp Asp Phe Thr Ala Glu Asp Leu
 85 90 95
 Lys Asp Leu Gly Glu Ile Gly Arg Gly Ala Tyr Gly Ser Val Asn Lys
 100 105 110
 Met Val His Lys Pro Ser Gly Gln Ile Met Ala Val Lys Arg Ile Arg
 115 120 125
 Ser Thr Val Asp Glu Lys Glu Gln Lys Gln Leu Leu Met Asp Leu Asp
 130 135 140
 Val Val Met Arg Ser Ser Asp Cys Pro Tyr Ile Val Gln Phe Tyr Gly
 145 150 155 160
 Ala Leu Phe Arg Glu Gly Asp Cys Trp Ile Cys Met Glu Leu Met Ser
 165 170 175
 Thr Ser Phe Asp Lys Phe Tyr Lys Tyr Val Tyr Ser Val Leu Asp Asp
 180 185 190
 Val Ile Pro Glu Glu Ile Leu Gly Lys Ile Thr Leu Ala Thr Val Lys
 195 200 205
 Ala Leu Met His Leu Lys Glu Asn Leu Lys Ile Ile His Arg Asp Ile
 210 215 220
 Lys Pro Ser Asn Ile Leu Leu Asp Arg Ser Gly Met Ile Lys Leu Cys
 225 230 235 240
 Asp Phe Gly Ile Ser Gly Gln Leu Val Asp Ser Ile Ala Lys Thr Arg
 245 250 255
 Asp Ala Gly Cys Arg Pro Tyr Met Ala Pro Glu Arg Ile Asp Phe Ser
 260 265 270
 Ala Ser Arg Gln Gly Tyr Asp Val Arg Ser Asp Val Trp Ser Leu Gly
 275 280 285
 Ile Thr Leu Tyr Glu Leu Ala Thr Gly Arg Phe Pro Tyr Pro Lys Trp
 290 295 300
 Asn Ser Val Phe Asp Gln Leu Thr Gln Val Val Lys Gly Asp Pro Pro
 305 310 315 320
 Gln Leu Ser Asn Ser Glu Glu Arg Glu Phe Ser Pro Ser Phe Ile Asn
 325 330 335
 Phe Val Asn Leu Cys Leu Thr Lys Asp Glu Ser Lys Arg Pro Lys Tyr
 340 345 350
 Lys Glu Leu Leu Lys His Pro Phe Ile Leu Met Tyr Glu Glu Arg Ala
 355 360 365
 Val Glu Val Ala Cys Tyr Val Cys Lys Ile Leu Asp Gln Met Pro Ala
 370 375 380
 Thr Pro Ser Ser Pro Met Tyr Val Asp
 385 390

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(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3576 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTCCCAACAA TGGCGGCTCC GAGCCCGAGC GGCGGCGGCG GCTCCGGGGG CGGCAGCGGC	60
AGCGGCACCC CCGGCCCCGT AGGGTCCCCG GCGCCAGGCC ACCCGGCCGT CAGCAGCATG	120
CAGGGTAAAC GCAAAGCACT GAAGTTGAAT TTTGCAAATC CACCTTTCAA ATCTACAGCA	180
AGGTTTACTC TGAATCCCAA TCCTACAGGA GTTCAAAACC CACACATAGA GAGACTGAGA	240
ACACACAGCA TTGAGTCATC AGGAAACTG AAGATCTCCC CTGAACAACA CTGGGATTTT	300
ACTGCAGAGG ACTTGAAAGA CCTTGGAGAA ATTGGACGAG GAGCTTATGG TTCTGTCAAC	360
AAAATGGTCC ACAAAACCAAG TGGGCAAATA ATGGCAGTTA AAAGAATTCT GTCAACAGTG	420
GATGAAAAAG AACAAAAACA ACTTCTTATG GATTTGGATG TAGTAATGCG GAGTAGTGAT	480
TGCCCATACA TTGTTCACTT TTATGGTGCA CTCTTCAGAG AGGGTGAAGT TTGGATCTGT	540
ATGGAAGTCA TGTCTACCTC GTTTGATAAG TTTTACAAAT ATGTATATAG TGTATTAGAT	600
GATGTTATTC CAGAAGAAAT TTTAGGCAAA ATCACTTTAG CAACTGTGAA AGCACTAAAC	660
CACTTAAAAG AAAACTTGAA AATTATTCAC AGAGATATCA AACCTTCCAA TATTCTTCTG	720
GACAGAAGTG GAAATATTAA GCTCTGTGAC TTCGGCATCA GTGGACAGCT TGTGGACTCT	780
ATTGCCAAGA CAAGAGATGC TGGCTGTAGG CCATACATGG CACCTGAAAG AATAGACCCA	840
AGCGCATCAC GACAAGGATA TGATGTCCGC TCTGATGTCT GGAGTTTGGG GATCACATTG	900
TATGAGTTGG CCACAGGCCG ATTTCCCTTAT CCAAAGTGGA ATAGTGTATT TGATCAACTA	960
ACACAAGTCG TGAAAGGAGA TCCTCCGCAG CTGAGTAATT CTGAGGAAAG GGAATTCTCC	1020
CCGAGTTTCA TCAACTTTGT CAAGTTGTGC CTTACGAAGG ATGAATCCAA AAGGCCAAAG	1080
TATAAAGAGC TTCTGAAACA TCCCTTTTATT TTGATGTATG AAGAACGTGC CGTTGAGGTC	1140
GCATGCTATG TTTGTAAAAT CCTGGATCAA ATGCCAGCTA CTCCCAGCTC TCCCATGTAT	1200
GTCGATTGAT ATCGCTGCTA CATCAGACTC TAGAAAAAAG GGCTGAGAGG AAGCAAGACG	1260
TAAAGAATTT TCATCCCGTA TCACAGTGTT TTTATTGCTC GCCCAGACAC CATGTGCAAT	1320
AAGATTGGTG TTCGTTTCCA TCATGTCTGT ATACTCCTGT CACCTAGAAC GTGCATCCTT	1380
GTAATACCTG ATTGATCACA CAGTGTTAGT GCTGGTCAGA GAGACCTCAT CCTGCTCTTT	1440
TGTGATGAAC ATATTCATGA AATGTGGAAG TCAGTACGAT CAAGTTGTTG ACTGTGATTA	1500
GATCACATCT TAAATTCATT TCTAGACTCA AAACCTGGAG ATGCAGCTAC TGGAAATGGTG	1560
TTTTGTCAGA CTTCCAAATC CTGGAAGGAC ACAGTGATGA ATGTACTATA TCTGAACATA	1620

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GAAACTCGGG	CTTGAGTGAG	AAGAGCTTGC	ACAGCCAACG	AGACACATTG	CCTTCTGGAG	1680
CTGGGAGACA	AAGGAGGAAT	TTACTTTCTT	CACCAAGTGC	AATAGATTAC	TGATGTGATA	1740
TTCTGTTGCT	TTACAGTTAC	AGTTGATGTT	TGGGGATCGA	TGTGCTCAGC	CAAATTCCT	1800
GTTTGAAATA	TCATGTTAAA	TTAGAAATGAA	TTTATCTTTA	CCAAAAACCA	TGTTGCGTTC	1860
AAAGAGGTGA	ACATTAAAAAT	ATAGAGACAG	GACAGAATGT	GTTCTTTTCT	CCTCTACCAG	1920
TCCTATTTTT	CAATGGGAAG	ACTCAGGAGT	CTGCCACTTG	TCAAAGAAGG	TGCTGATCCT	1980
AAGAATTTTT	CATTCTCAGA	ATTCGGTGTG	CTGCCAACTT	GATGTTCCAC	CTGCCACAAA	2040
CCACCAGGAC	TGAAGAAGA	AAACAGTACA	GAAGGCAAAG	TTTACAGATG	TTTTTAATTC	2100
TAGTATTTTA	TCTGGAACAA	CTTGTTAGCAG	CTATATATTT	CCCCTTGGTC	CCAAGCCTGA	2160
TACTTTAGCC	ATCATAACTC	ACTAACAGGG	AGAAGTAGCT	AGTAGCAATG	TGCCTTGATT	2220
GATTAGATAA	AGATTTCTAG	TAGGCAGCAA	AAGACCAAAT	CTCAGTTGTT	TGCTTCTTGC	2280
CATCACTGGT	CCAGGTCTTC	AGTTTCCGAA	TCTCTTTCCC	TTCCCCTGTG	GTCTATTGTC	2340
GCTATGTGAC	TTGCGCTTAA	TCCAATATTT	TGCCTTTTTT	CTATATCAAA	AAACCTTTAC	2400
AGTTAGCAGG	GATGTTCCCT	ACCGAGGATT	TTTAACCCCC	AATCTCTCAT	AATCGCTAGT	2460
GTTTAAAAGG	CTAAGAATAG	TGGGGCCCAA	CCGATGTGGT	AGGTGATAAA	GAGGCATCTT	2520
TTCTAGAGAC	ACATTGGACC	AGATGAGGAT	CCGAAACGGC	AGCCTTTACG	TTCATCACCT	2580
GCTAGAACCT	CTCGTAGTCC	ATCACCATT	CTTGGCATTG	GAATTCTACT	GGAAAAAAT	2640
ACAAAAAGCA	AAACAAAACC	CTCAGCACTG	TTACAAGAGG	CCATTTAAGT	ATCTTGTGCT	2700
TCTTCACTTA	CCCATTAGCC	AGGTTCTCAT	TAGGTTTTGC	TTGGGCCTCC	CTGGCACTGA	2760
ACCTTAGGCT	TTGTATGACA	GTGAAGCAGC	ACTGTGAGTG	GTTCAAGCAC	ACTGGAATAT	2820
AAAACAGTCA	TGGCCTGAGA	TGCAGGTGAT	GCCATTACAG	AACCAAATCG	TGGCACGTAT	2880
TGCTGTGTCT	CCTCTCAGAG	TGACAGTCAT	AAATACTGTC	AAACAATAAA	GGGAGAATGG	2940
TGCTGTTTAA	AGTCACATCC	CTGTAAATG	CAGAATTCAA	AAGTGATTAT	CTCTTTGATC	3000
TACTTGCCCTC	ATTTCCCTAT	CTTCTCCCCC	ACGGTATCCT	AAACTTTAGA	CTTCCCACTG	3060
TTCTGAAAGG	AGACATTGCT	CTATGTCTGC	CTTCGACCAC	AGCAAGCCAT	CATCCTCCAT	3120
TGCTCCCGGG	GACTCAAGAG	GAATCTGTTT	CTCTGCTGTC	AACTTCCCAT	CTGGCTCAGC	3180
ATAGGGTCAC	TTTGCCATTA	TGCAAATGGA	GATAAAAGCA	ATTCTGGCTG	TCCAGGAGCT	3240
AATCTGACCG	TTCTATTGTG	TGGATGACCA	CATAAGAAGG	CAATTTTAGT	GTATTAATCA	3300
TAGATTATTA	TAAACTATAA	ACTTAAGGGC	AAGGAGTTTA	TTACAATGTA	TCTTTATTAA	3360
AACAAAAGGG	TGTATAGTGT	TCACAAACTG	TGAAAATAGT	GTAAGAACTG	TACATTGTGA	3420
GCTCTGGTTA	TTTTTCTCTT	GTACCATAGA	AAAATGTATA	AAAATTATCA	AAAAGCTAAT	3480
GTGCAGGGAT	ATTGCCTTAT	TTGTCTGTAA	AAAATGGAGC	TCAGTAACAT	AACTGCTTCT	3540

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TGGAGCTTTG AATATTTTA TCCTGTATTC TTGTTT

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(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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Met Ala Ala Pro Ser Pro Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser
1          5          10          15
Gly Ser Gly Thr Pro Gly Pro Val Gly Ser Pro Ala Pro Gly His Pro
20          25          30
Ala Val Ser Ser Met Gln Gly Lys Arg Lys Ala Leu Lys Leu Asn Phe
35          40          45
Ala Asn Pro Pro Phe Lys Ser Thr Ala Arg Phe Thr Leu Asn Pro Asn
50          55          60
Pro Thr Gly Val Gln Asn Pro His Ile Glu Arg Leu Arg Thr His Ser
65          70          75          80
Ile Glu Ser Ser Gly Lys Leu Lys Ile Ser Pro Glu Gln His Trp Asp
85          90          95
Phe Thr Ala Glu Asp Leu Lys Asp Leu Gly Glu Ile Gly Arg Gly Ala
100         105         110
Tyr Gly Ser Val Asn Lys Met Val His Lys Pro Ser Gly Gln Ile Met
115         120         125
Ala Val Lys Arg Ile Arg Ser Thr Val Asp Glu Lys Glu Gln Lys Gln
130         135         140
Leu Leu Met Asp Leu Asp Val Val Met Arg Ser Ser Asp Cys Pro Tyr
145         150         155         160
Ile Val Gln Phe Tyr Gly Ala Leu Phe Arg Glu Gly Asp Cys Trp Ile
165         170         175
Cys Met Glu Leu Met Ser Thr Ser Phe Asp Lys Phe Tyr Lys Tyr Val
180         185         190
Tyr Ser Val Leu Asp Asp Val Ile Pro Glu Glu Ile Leu Gly Lys Ile
195         200         205
Thr Leu Ala Thr Val Lys Ala Leu Asn His Leu Lys Glu Asn Leu Lys
210         215         220
Ile Ile His Arg Asp Ile Lys Pro Ser Asn Ile Leu Leu Asp Arg Ser
225         230         235         240
Gly Asn Ile Lys Leu Cys Asp Phe Gly Ile Ser Gly Gln Leu Val Asp
245         250         255
Ser Ile Ala Lys Thr Arg Asp Ala Gly Cys Arg Pro Tyr Met Ala Pro
260         265         270

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Glu Arg Ile Asp Pro Ser Ala Ser Arg In Gly Tyr Asp Val Arg Ser
 275 280 285
 Asp Val Trp Ser Leu Gly Ile Thr Leu Tyr Glu Leu Ala Thr Gly Arg
 290 295 300
 Phe Pro Tyr Pro Lys Trp Asn Ser Val Phe Asp Gln Leu Thr Gln Val
 305 310 315 320
 Val Lys Gly Asp Pro Pro Gln Leu Ser Asn Ser Glu Glu Arg Glu Phe
 325 330 335
 Ser Pro Ser Phe Ile Asn Phe Val Asn Leu Cys Leu Thr Lys Asp Glu
 340 345 350
 Ser Lys Arg Pro Lys Tyr Lys Glu Leu Leu Lys His Pro Phe Ile Leu
 355 360 365
 Met Tyr Glu Glu Arg Ala Val Glu Val Ala Cys Tyr Val Cys Lys Ile
 370 375 380
 Leu Asp Gln Met Pro Ala Thr Pro Ser Ser Pro Met Tyr Val Asp
 385 390 395

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 393 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Pro Lys Lys Lys Pro Thr Pro Ile Gln Leu Asn Pro Ala Pro Asp
 1 5 10 15
 Gly Ser Ala Val Asn Gly Thr Ser Ser Ala Glu Thr Asn Leu Glu Ala
 20 25 30
 Leu Gln Lys Lys Leu Glu Glu Leu Glu Leu Asp Glu Gln Gln Arg Lys
 35 40 45
 Arg Leu Glu Ala Phe Leu Thr Gln Lys Gln Lys Val Gly Glu Leu Lys
 50 55 60
 Asp Asp Asp Phe Glu Lys Ile Ser Glu Leu Gly Ala Gly Asn Gly Gly
 65 70 75 80
 Val Val Phe Lys Val Ser His Lys Pro Ser Gly Leu Val Met Ala Arg
 85 90 95
 Lys Leu Ile His Leu Glu Ile Lys Pro Ala Ile Arg Asn Gln Ile Ile
 100 105 110
 Arg Glu Leu Gln Val Leu His Glu Cys Asn Ser Pro Tyr Ile Val Gly
 115 120 125
 Phe Tyr Gly Ala Phe Tyr Ser Asp Gly Glu Ile Ser Ile Cys Met Glu
 130 135 140
 His Met Asp Gly Gly Ser Leu Asp Gln Val Leu Lys Lys Ala Gly Arg
 145 150 155 160

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Ile Pro Glu Gln Ile Leu Gly Lys Val Ser Ile Ala Val Ile Lys Gly
 165 170 175
 Leu Thr Tyr Leu Arg Glu Lys His Lys Ile Met His Arg Asp Val Lys
 180 185 190
 Pro Ser Asn Ile Leu Val Asn Ser Arg Gly Glu Ile Lys Leu Cys Asp
 195 200 205
 Phe Gly Val Ser Gly Gln Leu Ile Asp Ser Met Ala Asn Ser Phe Val
 210 215 220
 Gly Thr Arg Ser Tyr Met Ser Pro Glu Arg Leu Gln Gly Thr His Tyr
 225 230 235 240
 Ser Val Gln Ser Asp Ile Trp Ser Met Gly Leu Ser Leu Val Glu Met
 245 250 255
 Ala Val Gly Arg Tyr Pro Ile Pro Pro Pro Asp Ala Lys Glu Leu Glu
 260 265 270
 Leu Met Phe Gly Cys Gln Val Glu Gly Asp Ala Ala Glu Thr Pro Pro
 275 280 285
 Arg Pro Arg Thr Pro Gly Arg Pro Leu Ser Ser Tyr Gly Met Asp Ser
 290 295 300
 Arg Pro Pro Met Ala Ile Phe Glu Leu Leu Asp Tyr Ile Val Asn Glu
 305 310 315 320
 Pro Pro Pro Lys Leu Pro Ser Gly Val Phe Ser Leu Glu Phe Gln Asp
 325 330 335
 Phe Val Asn Lys Cys Leu Ile Lys Asn Pro Ala Glu Arg Ala Asp Leu
 340 345 350
 Lys Gln Leu Met Val His Ala Phe Ile Lys Arg Ser Asp Ala Glu Glu
 355 360 365
 Val Asp Phe Ala Gly Trp Leu Cys Ser Thr Ile Gly Leu Asn Gln Pro
 370 375 380
 Ser Thr Pro Thr His Ala Ala Gly Val
 385 390

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 400 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Leu Ala Arg Arg Lys Pro Val Leu Pro Ala Leu Thr Ile Asn Pro
 1 5 10 15
 Thr Ile Ala Glu Gly Pro Ser Pro Thr Ser Glu Gly Ala Ser Glu Ala
 20 25 30
 Asn Leu Val Asp Leu Gln Lys Lys Leu Glu Glu Leu Glu Leu Asp Glu
 35 40 45

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Gln Gln Lys Lys Arg Leu Glu Ala Phe Leu Thr Gln Lys Ala Lys Val
 50 55 60
 Ser Glu Leu Lys Asp Asp Phe Glu Arg Ile Ser Glu Leu Gly Ala
 65 70 75 80
 Gly Asn Gly Gly Val Val Thr Lys Val Gln His Arg Pro Ser Gly Leu
 85 90 95
 Ile Met Ala Arg Lys Leu Ile His Leu Glu Ile Lys Pro Ala Ile Arg
 100 105 110
 Asn Gln Ile Ile Arg Glu Leu Gln Val Leu His Glu Cys Asn Ser Pro
 115 120 125
 Tyr Ile Val Gly Phe Tyr Gly Ala Phe Tyr Ser Asp Gly Glu Ile Ser
 130 135 140
 Ile Cys Met Glu His Met Asp Gly Gly Ser Leu Asp Gln Val Leu Lys
 145 150 155 160
 Glu Ala Lys Arg Ile Pro Glu Glu Ile Leu Gly Lys Val Ser Ile Ala
 165 170 175
 Val Leu Arg Gly Leu Ala Tyr Leu Arg Glu Lys His Gln Ile Met His
 180 185 190
 Arg Asp Val Lys Pro Ser Asn Ile Leu Val Asn Ser Arg Gly Glu Ile
 195 200 205
 Lys Leu Cys Asp Phe Gly Val Ser Gly Gln Leu Ile Asp Ser Met Ala
 210 215 220
 Asn Ser Phe Val Gly Thr Arg Ser Tyr Met Ala Pro Glu Arg Leu Gln
 225 230 235 240
 Gly Thr His Tyr Ser Val Gln Ser Asp Ile Trp Ser Met Gly Leu Ser
 245 250 255
 Leu Val Glu Leu Ala Val Gly Arg Tyr Pro Ile Pro Pro Pro Asp Ala
 260 265 270
 Lys Glu Leu Glu Ala Ile Phe Gly Arg Pro Val Val Asp Gly Glu Glu
 275 280 285
 Gly Glu Pro His Ser Ile Ser Pro Arg Pro Arg Pro Pro Gly Arg Pro
 290 295 300
 Val Ser Gly His Gly Met Asp Ser Arg Pro Ala Met Ala Ile Phe Glu
 305 310 315 320
 Leu Leu Asp Tyr Ile Val Asn Glu Pro Pro Pro Lys Leu Pro Asn Gly
 325 330 335
 Val Phe Thr Pro Asp Phe Gln Glu Phe Val Asn Lys Cys Leu Ile Lys
 340 345 350
 Asn Pro Ala Glu Arg Ala Asp Leu Lys Met Leu Thr Asn His Thr Phe
 355 360 365
 Ile Lys Arg Ser Glu Val Glu Glu Val Asp Phe Ala Gly Trp Leu Cys
 370 375 380

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Lys Thr Leu Arg Leu Asn Gln Pro Gly Thr Pro Thr Arg Thr Ala Val
 385 390 395 400

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 668 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Glu Asp Lys Phe Ala Asn Leu Ser Leu His Glu Lys Thr Gly Lys
 1 5 10 15

Ser Ser Ile Gln Leu Asn Glu Gln Thr Gly Ser Asp Asn Gly Ser Ala
 20 25 30

Val Lys Arg Thr Ser Ser Thr Ser His Tyr Asn Asn Ile Asn Ala
 35 40 45

Asp Leu His Ala Arg Val Lys Ala Phe Gln Glu Gln Arg Ala Leu Lys
 50 55 60

Arg Ser Ala Ser Val Gly Ser Asn Gln Ser Glu Gln Asp Lys Gly Ser
 65 70 75 80

Ser Gln Ser Pro Lys His Ile Gln Gln Ile Val Asn Lys Pro Leu Pro
 85 90 95

Pro Leu Pro Val Ala Gly Ser Ser Lys Val Ser Gln Arg Met Ser Ser
 100 105 110

Gln Val Val Gln Ala Ser Ser Lys Ser Thr Leu Lys Asn Val Leu Asp
 115 120 125

Asn Gln Glu Thr Gln Asn Ile Thr Asp Val Asn Ile Asn Ile Asp Thr
 130 135 140

Thr Lys Ile Thr Ala Thr Thr Ile Gly Val Asn Ile Gly Leu Pro Ala
 145 150 155 160

Thr Asp Ile Thr Pro Ser Val Ser Asn Thr Ala Ser Ala Thr His Lys
 165 170 175

Ala Gln Leu Leu Asn Pro Asn Arg Arg Ala Pro Arg Arg Pro Leu Ser
 180 185 190

Thr Gln His Pro Thr Arg Pro Asn Val Ala Pro His Lys Ala Pro Ala
 195 200 205

Ile Ile Asn Thr Pro Lys Gln Ser Leu Ser Ala Arg Arg Gly Leu Lys
 210 215 220

Leu Pro Pro Gly Gly Met Ser Leu Lys Met Pro Thr Lys Thr Ala Gln
 225 230 235 240

Gln Pro Gln Gln Phe Ala Pro Ser Pro Ser Asn Lys Lys His Ile Glu
 245 250 255

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Thr Leu Ser Asn Ser Lys Val Val Glu Gly Lys Arg Ser Asn Pro Gly
 260 265 270
 Ser Leu Ile Asn Gly Val Gln Ser Thr Ser Thr Ser Ser Ser Thr Glu
 275 280 285
 Gly Pro His Asp Thr Val Gly Thr Thr Pro Arg Thr Gly Asn Ser Asn
 290 295 300
 Asn Ser Ser Asn Ser Gly Ser Ser Gly Gly Gly Gly Leu Phe Ala Asn
 305 310 315 320
 Phe Ser Lys Tyr Val Asp Ile Lys Ser Gly Ser Leu Asn Phe Ala Gly
 325 330 335
 Lys Leu Ser Leu Ser Ser Lys Gly Ile Asp Phe Ser Asn Gly Ser Ser
 340 345 350
 Ser Arg Ile Thr Leu Asp Glu Leu Glu Phe Leu Asp Glu Leu Gly His
 355 360 365
 Gly Asn Tyr Gly Asn Val Ser Lys Val Leu His Lys Pro Thr Asn Val
 370 375 380
 Ile Met Ala Thr Lys Glu Val Arg Leu Glu Leu Asp Glu Ala Lys Phe
 385 390 395 400
 Arg Gln Ile Leu Met Glu Leu Glu Val Leu His Lys Cys Asn Ser Pro
 405 410 415
 Tyr Ile Val Asp Phe Tyr Gly Ala Phe Phe Ile Glu Gly Ala Val Tyr
 420 425 430
 Met Cys Met Glu Tyr Met Asp Gly Gly Ser Leu Asp Lys Ile Tyr Asp
 435 440 445
 Glu Ser Ser Glu Ile Gly Gly Ile Asp Glu Pro Gln Leu Ala Phe Ile
 450 455 460
 Ala Asn Ala Val Ile His Gly Leu Lys Glu Leu Lys Glu Gln His Asn
 465 470 475 480
 Ile Ile His Arg Asp Val Lys Pro Thr Asn Ile Leu Cys Ser Ala Asn
 485 490 495
 Gln Gly Thr Val Lys Leu Cys Asp Phe Gly Val Ser Gly Asn Leu Val
 500 505 510
 Ala Ser Leu Ala Lys Thr Asn Ile Gly Cys Gln Ser Tyr Met Ala Pro
 515 520 525
 Glu Arg Ile Lys Ser Leu Asn Pro Asp Arg Ala Thr Tyr Thr Val Gln
 530 535 540
 Ser Asp Ile Trp Ser Leu Gly Leu Ser Ile Leu Glu Met Ala Leu Gly
 545 550 555 560
 Arg Tyr Pro Tyr Pro Pro Glu Thr Tyr Asp Asn Ile Phe Ser Gln Leu
 565 570 575
 Ser Ala Ile Val Asp Gly Pro Pro Pro Arg Leu Pro Ser Asp Lys Phe
 580 585 590

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Ser Ser Asp Ala Gln Asp Phe Val Ser Leu Cys Leu Gln Lys Ile Pro
 595 600 605

Glu Arg Arg Pro Thr Tyr Ala Ala Leu Thr Glu His Pro Trp Leu Val
 610 615 620

Lys Tyr Arg Asn Gln Asp Val His Met Ser Glu Tyr Ile Thr Glu Arg
 625 630 635 640

Leu Glu Arg Arg Asn Lys Ile Leu Arg Glu Arg Gly Glu Asn Gly Leu
 645 650 655

Ser Lys Asn Val Pro Ala Leu His Met Gly Gly Leu
 660 665

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTYTAYGGNG CNTTYTTYAT HGA

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(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATBCTYTCNG GNGCCATKTA

20

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ASTYRYSASA SASASYS

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CLAIMS

What is claimed is:

1. A substantially pure human mitogen-activated protein kinase kinase (MKK) polypeptide having serine,
5 threonine, and tyrosine kinase activity, and phosphorylating human mitogen-activated protein (MAP) kinase p38.
2. A polypeptide of claim 1 comprising the amino acid sequence of SEQ ID NO:2.
- 10 3. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 2.
4. An isolated and purified polynucleotide sequence of claim 3 consisting of the sequence of SEQ ID NO:1 or degenerate variants thereof, or a polynucleotide
15 sequence fully complementary to the sequence of SEQ ID NO:1 or degenerate variants thereof.
5. An isolated and purified polynucleotide sequence of claim 3 consisting of a polynucleotide sequence that hybridizes under stringent hybridization
20 conditions to the sequence of SEQ ID NO:1.
6. A polypeptide of claim 1 comprising the amino acid sequence of SEQ ID NO:4.
7. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 6.
- 25 8. An isolated and purified polynucleotide sequence of claim 3 consisting of the sequence of SEQ ID NO:3 or degenerate variants thereof, or a polynucleotide

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sequence fully complementary to the sequence of SEQ ID NO:3 or degenerate variants thereof.

9. An isolated and purified polynucleotide sequence of claim 7 consisting of a polynucleotide
5 sequence that hybridizes under stringent hybridization conditions to the sequence of SEQ ID NO:3.

10. A polypeptide of claim 1, further characterized in that said polypeptide phosphorylates human mitogen-activated protein (MAP) kinase JNK.

10 11. A polypeptide of claim 10 comprising the amino acid sequence of SEQ ID NO:6.

12. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 11.

13. An isolated and purified polynucleotide
15 sequence of claim 12 consisting of the sequence of SEQ ID NO:5 or degenerate variants thereof, or a polynucleotide sequence fully complementary to the sequence of SEQ ID NO:5 or degenerate variants thereof.

14. An isolated and purified polynucleotide
20 sequence of claim 12 consisting of a polynucleotide sequence that hybridizes under stringent hybridization conditions to the sequence of SEQ ID NO:5.

15. A polypeptide of claim 10 comprising an amino acid sequence of SEQ ID NO:8.

25 16. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 15.

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17. An isolated and purified polynucleotide sequence of claim 16 consisting of the sequence of SEQ ID NO:7 or degenerate variants thereof, or a polynucleotide sequence fully complementary to the sequence of SEQ ID
5 NO:7 or degenerate variants thereof.

18. An isolated and purified polynucleotide sequence of claim 16 consisting of a polynucleotide sequence that hybridizes under stringent hybridization conditions to the sequence of SEQ ID NO:7.

10 19. A polypeptide of claim 10 comprising the amino acid sequence of SEQ ID NO:10.

20. An isolated and purified polynucleotide sequence encoding a polypeptide of claim 19.

21. An isolated and purified polynucleotide
15 sequence of claim 20 consisting of the sequence of SEQ ID NO:9 or degenerate variants thereof, or a polynucleotide sequence fully complementary to the sequence of SEQ ID NO:9 or degenerate variants thereof.

22. A recombinant expression vector comprising a
20 polynucleotide sequence of any one of claims 3, 7, 12, 16, or 20.

23. A recombinant host cell comprising a polynucleotide sequence of any one of claims 3, 7, 12, 16, or 20.

25 24. A purified antibody which binds specifically to a polypeptide of any one of claims 1, 2, 6, 10, 11, 15, or 19.

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25. A method of measuring the activity of a mitogen-activated protein kinase kinase (MKK) in a biological test sample, said method comprising:

- a) incubating said test sample with an MKK
5 substrate for the MKK polypeptide of claim 1 and labeled phosphate under conditions sufficient to allow phosphorylation of said substrate, and
- b) determining the rate of incorporation of
10 labeled phosphate into said substrate, wherein said rate of incorporation is a measure of MKK activity.

26. A method of claim 25, wherein said MKK substrate is selected from the group consisting of p38 and JNK MAP kinases, activating transcription factor-2 (ATF2), ATFa, cAMP response element binding protein (CRE-
15 BPa), and c-Jun.

27. A method of claim 25, wherein said biological test sample is fluid, cells, or tissue obtained from a mammal.

28. A method for measuring the synthesis of MKK
20 in a biological test sample, comprising the steps of:
a) fractionating proteins present in said sample by gel electrophoresis;
b) transferring the proteins onto a membrane; and
c) probing the proteins with a labeled antibody
25 specific to a MKK polypeptide of claim 1, wherein the level of MKK synthesis is determined by the amount of bound labeled antibody.

29. A method for measuring the level of expression of MKK in a test sample, comprising the steps
30 of:

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a) isolating polyadenylated RNA from the test sample;

b) incubating polyadenylated RNA with a polynucleotide probe specific for a MKK polypeptide of claim 1;

c) determining the amount of said probe hybridized said polyadenylated RNA, wherein the level of expression of MKK is directly related to the amount of MKK probe hybridized to said RNA.

10 30. A method for identifying a reagent which modulates MKK synthesis, said method comprising:

a) using the method of claim 28;

b) comparing the effect of said reagent on MKK synthesis relative to a control, wherein a reagent able to modulate MKK synthesis is identified.

31. A method of claim 30 wherein said MKK substrate is one or more of p38, JNK, ATF2, ATFα, CRE-BPa, and c-Jun.

32. A method of claim 30 wherein said modulation is inhibition of MKK synthesis.

33. A substantially pure human mitogen-activated protein kinase kinase (MKK) polypeptide of any one of claims 1, 2, 6, 10, 11, 15, or 19 for use in treating an MKK-mediated disorder.

25 34. The polypeptide of claim 33, wherein said MKK-mediated disorder is selected from the group consisting of ischemic heart disease, kidney failure, oxidative liver damage, respiratory distress syndrome, heat and radiation burns, septic shock, rheumatoid

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arthritis, autoimmune disorders, and inflammatory diseases.

35. The use of a polypeptide of claim 33 for the manufacture of a medicament for the treatment of an MKK-
5 mediated disorder.

36. A kit useful for the detection of MKK, said kit comprising a buffer and a reagent which binds to a MKK polypeptide of any one of claims 1, 2, 6, 10, 11, 15, or 19, wherein a sample to be tested is mixed with said
10 buffer and said reagent, and wherein said reagent is labeled.

37. A kit of claim 36, wherein said reagent is an antibody that specifically binds MKK.

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WIS1  MSPP-----APPTPPPS-----LSPTTITG-----GRTVEKAG
WIS4  MCKKALALDPAH  FRTARPTLH  ...GVD  FRIERLT...  SCKL  SP-----GRTVPTL
WIS1  MSPP--TPICLH  A  PCKLAVCTSSAETHLALORLEEL  ...  CKKALALFTRORV  LAG
WIS2  FLARRPVLPAITR  TLACSPPTSCASALVLOKLEEL  ...  CKKALALFTRORV  LAG
PBS2  GTTPPTONHNS  -GKSGCGGLFANTRTVDSKSLNFACT  ...  SRS  GRTS-----GRTVPTL
Consensus
12      I      II      III      IV      142
WIS1  LVTISLNGATGVVERVNAQCT  I  NAVE  I  RATVWSOCOL  ...  LKLDINHTVOCFTT  VTTICAL/RS
WIS4  LKLG  I  ...  S  H  NV  SP  ...  O  ...  S  ...  DEK  ...  C  ...  VV  ...  SS  ...  P  I  O  ...
WIS1  FES  ...  A  SG  ...  F  VS  SP  ...  LV  ...  R  L  SLKIPAINC  ...  I  L  QV  ...  LKES  ...  S  G  ...  FTS
WIS2  FES  ...  A  SG  ...  T  VQ  SP  ...  L  ...  R  L  SLKIPAINC  ...  I  L  QV  ...  LKES  ...  S  G  ...  FTS
PBS2  FES  ...  S  H  ...  S  VL  RPTV  ...  T  EV  ...  LKLG  ...  FRT  ...  I  L  QV  ...  LKES  ...  S  G  ...  FTS
Consensus  C  C  C  G  V  E  H  ...  R  A  E  ...  L  ...  F  V  F  T  C  A  G
143      V      VI      212
WIS1  PWTCHLAP  T  LKRT  ---  EVLSKQWTFES  I  L  G  I  A  V  T  ...  T  A  L  K  L  A  S  L  V  T  H  R  V  P  S  V  L  ...
WIS4  C  ...  S  ...  F  ...  RTV  ...  S  ...  E  ...  T  L  A  S  ...  S  ...  R  E  K  ...  I  ...  I  ...  L  ...
WIS1  S  I  S  ...  S  ...  G  ...  F  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...
WIS2  S  I  S  ...  S  ...  G  ...  F  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...
PBS2  A  T  H  ...  T  ...  G  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...
Consensus  C  H  E  S  S  O  ...  I  S  L  ...  L  ...  S  R  S  S  S  L
213      VII      VIII      IX      294
WIS1  SCVVICMG  I  S  C  T  L  V  S  V  A  T  H  A  C  K  P  T  H  A  P  E  R  I  N  T  ...  S  L  A  C  T  V  T  V  S  V  L  S  I  T  H  A  I  L  A  P  T  ...
WIS4  S  S  I  L  ...  O  ...  I  ...  R  ...  S  ...  S  ...  S  A  S  G  ...  S  ...  S  ...  L  ...  L  ...  T  ...
WIS1  S  S  I  L  ...  V  ...  O  ...  I  ...  R  ...  S  ...  S  ...  S  A  S  G  ...  S  ...  S  ...  L  ...  L  ...  T  ...
WIS2  S  S  I  L  ...  V  ...  O  ...  I  ...  R  ...  S  ...  S  ...  S  A  S  G  ...  S  ...  S  ...  L  ...  L  ...  T  ...
PBS2  Q  T  L  ...  V  ...  A  ...  L  ...  S  ...  S  ...  S  ...  S  A  S  G  ...  S  ...  S  ...  L  ...  L  ...  T  ...
Consensus  G  S  C  V  S  S  C  L  S  A  ...  G  T  H  P  E  R  ...  T  V  S  S  S  S  C  S  A  S  P
295      X      355
WIS1  L  S  G  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...
WIS4  P  E  S  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...
WIS1  P  A  S  S  E  L  A  I  F  C  R  P  V  V  E  E  E  L  F  S  I  S  P  R  A  P  C  R  V  V  E  K  H  E  L  A  I  A  N  A  I  ...  S  L  ...  S  ...  S  ...  S  ...
WIS2  P  A  S  S  E  L  A  I  F  C  R  P  V  V  E  E  E  L  F  S  I  S  P  R  A  P  C  R  V  V  E  K  H  E  L  A  I  A  N  A  I  ...  S  L  ...  S  ...  S  ...  S  ...
PBS2  T  T  S  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...
Consensus  F  L  V  P  L
356      XI      426
WIS1  F  S  P  V  V  T  A  C  L  A  S  P  A  S  G  T  L  E  M  E  R  P  T  T  L  A  N  T  K  T  S  I  A  A  F  V  ...  ...  ...  ...
WIS4  ...  S  ...  S  ...  V  E  L  ...  T  ...  S  E  R  ...  P  E  S  ...  L  E  ...  ...  ...  ...  ...  ...  ...  ...  ...
WIS1  ...  L  ...  G  ...  V  H  E  ...  I  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...
WIS2  ...  T  ...  S  ...  G  ...  V  H  E  ...  I  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...  ...
PBS2  ...  S  ...  A  ...  G  ...  V  E  L  ...  O  ...  S  ...  S  ...  E  ...  P  ...  T  ...  A  ...  A  ...  T  ...  ...  ...  ...  ...  ...
Consensus  F  F  C  L  S  S  L  S

```

FIG. 1

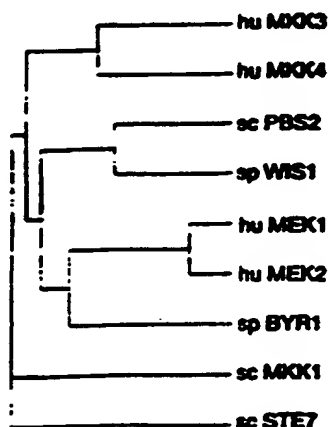


FIG. 2

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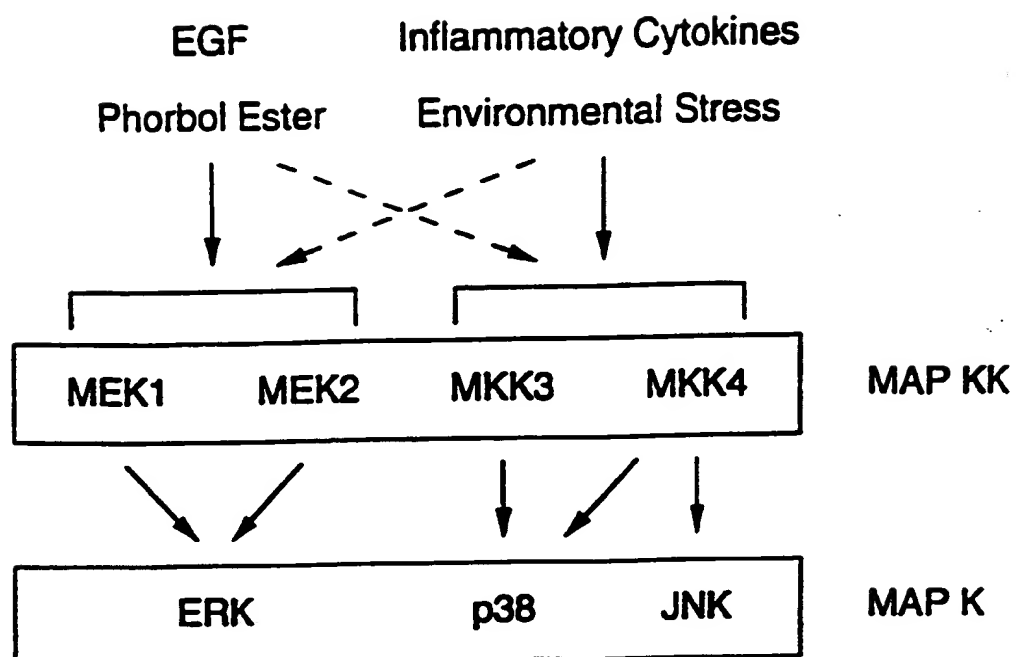


FIG. 3

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FIG. 4

```

      5   10   15   20   25   30   35   40   45   50   55   60
      *   *   *   *   *   *   *   *   *   *   *   *
TGGCTGGCAA TGGCCTTGCT GACCTCGAGC CGGGCCCCACG TGGGGACCTT TGGAGCACAG
ACCGACCGTT ACCGGAACGA CTGGAGCTCG GCCCGGGTGC ACCCTTGAA ACCTCGTGTC

      65   70   75   80   85   90   95  100  105  110  115  120
      *   *   *   *   *   *   *   *   *   *   *   *
CCTACGATCC TGGTGCAAGG CCGGTGGATG CAGAGGCCAG TCCATATACC ACCCAGGCCT
GGATGCTAGG ACCACGTTCC GGCCACCTAC GTCTCCGGTC AGGTATATGG TGGGTCCGGA

      125  130  135  140  145  150  155  160  165  170  175  180
      *   *   *   *   *   *   *   *   *   *   *   *
GCGAGGAGCG TGGTCCCCAC CCATCCAGCC CATATGTGCA AGTGCCCTTG ACAGAGAGCC
GGCTCCTCGC ACCAGGGGTG GGTAGGTCGG GTATACACGT TCACGGGAAC TGTCTCTCCG

      185  190  195  200  205  210  215  220  225  230  235  240
      *   *   *   *   *   *   *   *   *   *   *   *
TGGTCATATC CATGGTGACC ATTTATGGGC CACAACAGGT CCCCATCTGC GCAGTGAACC
ACCAATATAG GTACCACTGG TAAATACCCG GTGTTGTCCA GGGGTAGACG CGTCACTTGG

      245  250  255  260  265  270  275  280  285  290  295  300
      *   *   *   *   *   *   *   *   *   *   *   *
CTGTGCTGAG CACCTTGACG ACGTGATCTT GCTTCGTCTCT GCAGCACTGT GCGGGGCAGG
GACACGACTC GTGGAACGTC TGCACTAGAA CGAAGCAGGA CGTCGTGACA CGCCCCGTCC

      305  310  315  320  325  330  335  340  345  350  355
      *   *   *   *   *   *   *   *   *   *   *
AAAATCCAAG AGGAAGAAGG ATCTACGGAT ATCTCTGC ATG TCC AAG CCA CCC GCA
TTTTAGGTTC TCCTTCTTCC TAGATGCCTA TAGGACG TAC AGG TTC GGT GGG CGT
                                   Met Ser Lys Pro Pro Ala>

      360  365  370  375  380  385  390  395  400
      *   *   *   *   *   *   *   *   *
CCC AAC CCC ACA CCC CCC CGG AAC CTG GAC TCC CGG ACC TTC ATC ACC
GGG TTG GGG TGT GGG GGG GCC TTG GAC CTG AGG GCC TGG AAG TAG TGG
Pro Asn Pro Thr Pro Pro Arg Asn Leu Asp Ser Arg Thr Phe Ile Thr>

405   410   415   420   425   430   435   440   445   450
      *   *   *   *   *   *   *   *   *   *
ATT GGA GAC AGA AAC TTT GAG GTG GAG GCT GAT GAC TTG GTG ACC ATC
TAA CCT CTG TCT TTG AAA CTC CAC CTC CGA CTA CTG AAC CAC TGG TAG
Ile Gly Asp Arg Asn Phe Glu Val Glu Ala Asp Asp Leu Val Thr Ile>

      455  460  465  470  475  480  485  490  495
      *   *   *   *   *   *   *   *   *
TCA GAA CTG GGC CGT GGA GCC TAT GGG GTG GTA GAG AAG GTG CGG CAC
AGT CTT GAC CCG GCA CCT CGG ATA CCC CAC CAT CTC TTC CAC GCC GTG
Ser Glu Leu Gly Arg Gly Ala Tyr Gly Val Val Glu Lys Val Arg His>

500   505   510   515   520   525   530   535   540   545
      *   *   *   *   *   *   *   *   *   *
GCC CAG AGC GGC ACC ATC ATG GCC GTG AAG CGG ATC CGG GCC ACC GTG
CGG GTC TCG CCG TGG TAG TAC CGG CAC TTC GCC TAG GCC CGG TGG CAC
Ala Gln Ser Gly Thr Il Met Ala Val Lys Arg Ile Arg Ala Thr Val>

      550   555   560   565   570   575   580   585   590   595
      *   *   *   *   *   *   *   *   *   *
AAC TCA CAG GAG CAG AAG CGG CTG CTC ATG GAC CTG GAC ATC AAC ATG
TTG AGT GTC CTC GTC TTC GCC GAC GAG TAC CTG GAC CTG TAG TTG TAC
Asn Ser Gln Glu Gln Lys Arg Leu Leu Met Asp Leu Asp Ile Asn Met>

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FIG. 4 - CONT'D

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        600      605      610      615      620      625      630      635      640
        *        *        *        *        *        *        *        *        *
CGC ACG GTC GAC TGT TTC TAC ACT GTC ACC TTC TAC GGG GCA CTA TTC
GCG TGC CAG CTG ACA AAG ATG TGA CAG TGG AAG ATG CCC CGT GAT AAG
Arg Thr Val Asp Cys Phe Tyr Thr Val Thr Phe Tyr Gly Ala Leu Phe>

645      650      655      660      665      670      675      680      685      690
        *        *        *        *        *        *        *        *        *
AGA GAG GGA GAC GTG TGG ATC TGC ATG GAG CTC ATG GAC ACA TCC TTG
TCT CTC CCT CTG CAC ACC TAG ACG TAC CTC GAG TAC CTG TGT AGG AAC
Arg Glu Gly Asp Val Trp Ile Cys Met Glu Leu Met Asp Thr Ser Leu>

        695      700      705      710      715      720      725      730      735
        *        *        *        *        *        *        *        *        *
GAC AAG TTC TAC CGG AAG GTG CTG GAT AAA AAC ATG ACA ATT CCA GAG
CTG TTC AAG ATG GCC TTC CAC GAC CTA TTT TTG TAC TGT TAA GGT CTC
Asp Lys Phe Tyr Arg Lys Val Leu Asp Lys Asn Met Thr Ile Pro Glu>

740      745      750      755      760      765      770      775      780      785
        *        *        *        *        *        *        *        *        *
GAC ATC CTT GGG GAG ATT GCT GTG TCT ATC GTG CGG GCC CTG GAG CAT
CTG TAG GAA CCC CTC TAA CGA CAC AGA TAG CAC GCC CGG GAC CTC GTA
Asp Ile Leu Gly Glu Ile Ala Val Ser Ile Val Arg Ala Leu Glu His>

790      795      800      805      810      815      820      825      830      835
        *        *        *        *        *        *        *        *        *
CTG CAC AGC AAG CTG TCG GTG ATC CAC AGA GAT GTG AAG CCC TCC AAT
GAC GTG TCG TTC GAC AGC CAC TAG GTG TCT CTA CAC TTC GGG AGG TTA
Leu His Ser Lys Leu Ser Val Ile His Arg Asp Val Lys Pro Ser Asn>

        840      845      850      855      860      865      870      875      880
        *        *        *        *        *        *        *        *        *
GTC CTT ATC AAC AAG GAG GGC CAT GTG AAG ATG TGT GAC TTT GGC ATC
CAG GAA TAG TTG TTC CTC CCG GTA CAC TTC TAC ACA CTG AAA CCG TAG
Val Leu Ile Asn Lys Glu Gly His Val Lys Met Cys Asp Phe Gly Ile>

885      890      895      900      905      910      915      920      925      930
        *        *        *        *        *        *        *        *        *
AGT GGC TAC TTG GTG GAC TCT GTG GCC AAG ACG ATG GAT GCC GGC TGC
TCA CCG ATG AAC CAC CTG AGA CAC CGG TTC TGC TAC CTA CGG CCG ACG
Ser Gly Tyr Leu Val Asp Ser Val Ala Lys Thr Met Asp Ala Gly Cys>

        935      940      945      950      955      960      965      970      975
        *        *        *        *        *        *        *        *        *
AAG CCC TAC ATG GCC CCT GAG AGG ATC AAC CCA GAG CTG AAC CAG AAG
TTC GGG ATG TAC CGG GGA CTC TCC TAG TTG GGT CTC GAC TTG GTC TTC
Lys Pro Tyr Met Ala Pro Glu Arg Ile Asn Pro Glu Leu Asn Gln Lys>

980      985      990      995      1000      1005      1010      1015      1020      1025
        *        *        *        *        *        *        *        *        *
GGC TAC AAT GTC AAG TCC GAC GTC TGG AGC CTG GGC ATC ACC ATG ATT
CCG ATG TTA CAG TTC AGG CTG CAG ACC TCG GAC CCG TAG TGG TAC TAA
Gly Tyr Asn Val Lys Ser Asp Val Trp Ser Leu Gly Ile Thr Met Ile>

1030      1035      1040      1045      1050      1055      1060      1065      1070      1075
        *        *        *        *        *        *        *        *        *
GAG ATG GCC ATC CTG CCG TTC CCT TAC GAG TCC TGG GGG ACC CCG TTC
CTC TAC CGG TAG GAC GCC AAG GGA ATG CTC AGG ACC CCC TGG GGC AAG
Glu Met Ala Ile Leu Arg Phe Pro Tyr Glu Ser Trp Gly Thr Pro Phe>

1080      1085      1090      1095      1100      1105      1110      1115      1120

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FIG. 4 - CONT'D

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      *           *           *           *           *
CAG CAG CTG AAG CAG GTG GTG GAG GAG CCG TCC CCC CAG CTC CCA GCC
GTC GTC GAC TTC GTC CAC CAC CTC CTC GGC AGG GGG GTC GAG GGT CGG
Gln Gln Leu Lys Gln Val Val Glu Glu Pro Ser Pro Gln Leu Pro Ala>

1125  1130  1135  1140  1145  1150  1155  1160  1165  1170
      *           *           *           *           *
GAC CGT TTC TCC CCC GAG TTT GTG GAC TTC ACT GCT CAG TGC CTG AGG
CTG GCA AAG AGG GGG CTC AAA CAC CTG AAG TGA CGA GTC ACG GAC TCC
Asp Arg Phe Ser Pro Glu Phe Val Asp Phe Thr Ala Gln Cys Leu Arg>

1175  1180  1185  1190  1195  1200  1205  1210  1215
      *           *           *           *           *
AAG AAC CCC GCA GAG CGT ATG AGC TAC CTG GAG CTG ATG GAG CAC CCC
TTC TTG GGG CGT CTC GCA TAC TCG ATG GAC CTC GAC TAC CTC GTG GGG
Lys Asn Pro Ala Glu Arg Met Ser Tyr Leu Glu Leu Met Glu His Pro>

1220  1225  1230  1235  1240  1245  1250  1255  1260  1265
      *           *           *           *           *
TTC TTC ACC TTG CAC AAA ACC AAG AAG ACG GAC ATT GCT GCC TTC GTG
AAG AAG TGG AAC GTG TTT TGG TTC TTC TGC CTG TAA CGA CGG AAG CAC
Phe Phe Thr Leu His Lys Thr Lys Lys Thr Asp Ile Ala Ala Phe Val>

1270  1275  1280  1285  1290  1295  1300  1305  1310  1315  1320
      *           *           *           *           *
AAG AAG ATC CTG GGA GAA GAC TCA TAGGGGCTG GGCTCCGGAC CCCACTCCGG
TTC TTC TAG GAC CCT CTT CTG AGT ATCCCGAC CCGGAGCCTG GGGTGAGGCC
Lys Lys Ile Leu Gly Glu Asp Ser> (SEQ ID NO:2)

1325  1330  1335  1340  1345  1350  1355  1360  1365  1370  1375  1380
      *           *           *           *           *
CCCTCCAGAG CCCACAGCC CCATCTGCGG GGGCAGTGCT CACCCACACC ATAAGCTACT
GGGAGGTCTC GGGGTGTCCG GGTAGACGCC CCGTCACTGA GTGGGTGTGG TATTGATGA

1385  1390  1395  1400  1405  1410  1415  1420  1425  1430  1435  1440
      *           *           *           *           *
GCCATCTCTG CCCAGGGCAT CTGGGAGGAA CCGAGGGGGC TGCTCCACCC TGGCTCTGTG
CGGTAGGACC GGGTCCCGTA GACCTCTCTT GGCTCCCCCG ACCAGGGTGG ACCGAGACAC

1445  1450  1455  1460  1465  1470  1475  1480  1485  1490  1495  1500
      *           *           *           *           *
GCGAGCCATT TGTCCCAAGT GCCAAGAAG CAGACCATTG GGGCTCCAG CCAGGCCCTT
CGCTCGGTAA ACAGGGTTCA CGGTTCCTTC GTCTGGTAAC CCGAGGGGTC GGTCCGGGAA

1505  1510  1515  1520  1525  1530  1535  1540  1545  1550  1555  1560
      *           *           *           *           *
GTCGGCCCCA CCAGTGCCTC TCCCTGCTGC TCCTAGGACC CGTCTCCAGC TGCTGAGATC
CAGCCGGGGT GGTACCGGAG AGGGACGACG AGGATCCTGG GCAGAGGTCTG ACCACTCTAG

1565  1570  1575  1580  1585  1590  1595  1600  1605  1610  1615  1620
      *           *           *           *           *
CTGGACTGAG GGGGCCTGGA TGCCCCCTGT GGATGCTGCT GCGCTGCAC AGCAGGCTGC
GACCTGACTC CCGCGGACCT ACGGGGACAA CCTACGACGA CCGGGACGTG TCGTCCGACG

1625  1630  1635  1640  1645  1650  1655  1660  1665  1670  1675  1680
      *           *           *           *           *
CAGTGCCTGG GTGGATGGGC CACCGCCTTG CCCAGCCTGG ATGCCATCCA AGTGTATAT
GTCACGGACC CACCTACCGG GTGGCGGAAC GGGTCGGACC TACGGTAGGT TCAACATATA

1685  1690  1695  1700  1705  1710  1715  1720  1725  1730  1735  1740
      *           *           *           *           *
TTTTTTAATC TCTCGACTGA ATGGACTTTG CACACTTTGG CCCAGGGTGG CCACACCTCT

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FIG. 4 - CONT'D

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AAAAAATTAG AGAGCTGACT TACCTGAAAC GTGTGAAACC GGGTCCCACC GGTGTGGAGA
1745 1750 1755 1760 1765 1770 1775 1780 1785 1790 1795 1800
* * * * *
ATCCCGGCTT TGGTGC GGGG TACACAAGAG GGGATGAGTT GTGTGAATAC CCCAAGACTC
TAGGGCCGAA ACCACGCCCC ATGTGTTCTC CCCTACTCAA CACACTTATG GGGTTCTGAG
1805 1810 1815 1820 1825 1830 1835 1840 1845 1850 1855 1860
* * * * *
CCATGAGGGA GATGCCATGA GCCGCCCAAG GCCTTCCCCT GGCAGTGGCA AACAGGGCCT
GGTACTCCCT CTACGGTACT CGGCGGGTTC CGGAAGGGGA CCGTGACCGT TTGTCCCGGA
1865 1870 1875 1880 1885 1890 1895 1900 1905 1910 1915 1920
* * * * *
CTGCGGAGCA CACTGGCTCA CCCAGTCCTG CCCGCCACCG TTATCGGTGT CATTACCTT
GACGCCCTCGT GTGACCGAGT GGGTCAGGAC GGGCGGTGGC AATAGCCACA GTAAGTGGAA
1925 1930 1935 1940 1945 1950 1955 1960 1965 1970 1975 1980
* * * * *
TCGTGTTTTT TTTAATTTAT CCTCTGTTGA TTTTTCCTTT TGCTTTATGG GTTTGGCTTG
AGCACAAAAA AAATTAAATA GGAGACAAC TAAAAAGAAA ACGAAATACC CAAACCGAAC
1985 1990 1995 2000 2005 2010 2015 2020 2025 2030
* * * * *
TTTTTCTTGC ATGGTTTGGA GCTGATCGCT TCTCCCCAC CCCCTAGGGG (SEQ ID NO: 1)
AAAAAGAACG TACCAAACCT CGACTAGCGA AGAGGGGGTG GGGGATCCCC

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FIG. 5

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      5   10   15   20   25   30   35   40   45   50   55   60
      *   *   *   *   *   *   *   *   *   *   *   *
TAGCTGCAGC ACAGCCTTCC CTAACGTTGC AACTGGGGGA AAAATCACTT TCCAGTCTGT
ATCGACGTCG TGTCGGAAGG GATTGCAACG TTGACCCCTT TTTAGTGAA AGGTCAGACA

      65   70   75   80   85   90   95  100  105  110  115  120
      *   *   *   *   *   *   *   *   *   *   *   *
TTTGCAAGGT GTGCATTTC ATCTTGATTG CCTGAAAGTC CATCTGCTGC ATCGGTCAAG
AAACGTTCCA CACGTAAAGG TAGAACTAAG GGACTTTCAG GTAGACGACG TAGCCAGTTC

      125  130  135  140  145  150  155  160  165  170  175  180
      *   *   *   *   *   *   *   *   *   *   *   *
AGAAACTCCA CTGTCATGAA GATTGCACGC CTGCAGCTTG CATCTTTGTT GCAAACTAG
TCTTTGAGGT GAACGTACTT CTAACGTGCG GACGTCGAAC GTAGAAACAA CGTTTGTATC

      185  190  195  200  205  210  215  220  225  230  235  240
      *   *   *   *   *   *   *   *   *   *   *   *
CTACAGAAGA GAAGCAAGGC AAAGTCTTTT GTGCTCCCTT CCCCATCAA AGGAAAGGGG
GATGTCCTCT CTTCGTTCCG TTTCAGAAAA CACGAGGGGA GGGGGTAGTT TCCTTTCCCT

      245  250  255  260  265  270  275  280  285
      *   *   *   *   *   *   *   *   *
AAA ATG TCT CAG TCG AAA GGC AAG AAG CGA AAC CCT GGC CTT AAA ATT
TTT TAC AGA GTC AGC TTT CCG TTC TTC GCT TTG GGA CCG GAA TTT TAA
Met Ser Gln Ser Lys Gly Lys Lys Arg Asn Pro Gly Leu Lys Ile>

290  295  300  305  310  315  320  325  330  335
      *   *   *   *   *   *   *   *   *   *
CCA AAA GAA GCA TTT GAA CAA CCT CAG ACC AGT TCC ACA CCA CCT AGA
GGT TTT CTT CGT AAA CTT GTT GGA GTC TGG TCA AGG TGT GGT GGA TCT
Pro Lys Glu Ala Phe Glu Gln Pro Gln Thr Ser Ser Thr Pro Pro Arg>

      340  345  350  355  360  365  370  375  380
      *   *   *   *   *   *   *   *   *
GAT TTA GAC TCC AAG GCT TGC ATT TCT ATT GGA AAT CAG AAC TTT GAG
CTA AAT CTG AGG TTC CGA ACG TAA AGA TAA CCT TTA GTC TTG AAA CTC
Asp Leu Asp Ser Lys Ala Cys Ile Ser Ile Gly Asn Gln Asn Phe Glu>

385  390  395  400  405  410  415  420  425  430
      *   *   *   *   *   *   *   *   *   *
GTG AAG GCA GAT GAC CTG GAG CCT ATA ATG GAA CTG GGA CGA GGT GCG
CAC TTC CGT CTA CTG GAC CTC GGA TAT TAC CTT GAC CCT GCT CCA CGC
Val Lys Ala Asp Asp Leu Glu Pro Ile Met Glu Leu Gly Arg Gly Ala>

      435  440  445  450  455  460  465  470  475  480
      *   *   *   *   *   *   *   *   *   *
TAC GGG GTG GTG GAG AAG ATG CGG CAC GTG CCC AGC GGG CAG ATC ATG
ATG CCC CAC CAC CTC TTC TAC GCC GTG CAC GGG TCG CCC GTC TAG TAC
Tyr Gly Val Val Glu Lys Met Arg His Val Pro Ser Gly Gln Ile Met>

      485  490  495  500  505  510  515  520  525
      *   *   *   *   *   *   *   *   *
GCA GTG AAG CGG ATC CGA GCC ACA GTA AAT AGC CAG GAA CAG AAA CGG
CGT CAC TTC GCC TAG GCT CGG TGT CAT TTA TCG GTC CTT GTC TTT GCC
Ala Val Lys Arg Ile Arg Ala Thr Val Asn Ser Gln Glu Gln Lys Arg>

530  535  540  545  550  555  560  565  570  575
      *   *   *   *   *   *   *   *   *   *
CTA CTG ATG GAT TTG GAT ATT TCC ATG AGG ACG GTG GAC TGT CCA TTC
GAT GAC TAC CTA AAC CTA TAA AGG TAC TCC TGC CAC CTG ACA GGT AAG

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FIG. 5 - CONT'D

Leu Leu Met Asp Leu Asp Ile Ser Met Arg Thr Val Asp Cys Pro Phe>
 580 585 590 595 600 605 610 615 620
 ACT GTC ACC TTT TAT GGC GCA CTG TTT CCG GAG GGT GAT GTG TGG ATC
 TGA CAG TGG AAA ATA CCG CGT GAC AAA GCC CTC CCA CTA CAC ACC TAG
 Thr Val Thr Phe Tyr Gly Ala Leu Phe Arg Glu Gly Asp Val Trp Ile>
 625 630 635 640 645 650 655 660 665 670
 TGC ATG GAG CTC ATG GAT ACA TCA CTA GAT AAA TTC TAC AAA CAA GTT
 ACG TAC CTC GAG TAC CTA TGT AGT GAT CTA TTT AAG ATG TTT GTT CAA
 Cys Met Glu Leu Met Asp Thr Ser Leu Asp Lys Phe Tyr Lys Gln Val>
 675 680 685 690 695 700 705 710 715 720
 ATT GAT AAA GGC CAG ACA ATT CCA GAG GAC ATC TTA GGG AAA ATA GCA
 TAA CTA TTT CCG GTC TGT TAA GGT CTC CTG TAG AAT CCC TTT TAT CGT
 Ile Asp Lys Gly Gln Thr Ile Pro Glu Asp Ile Leu Gly Lys Ile Ala>
 725 730 735 740 745 750 755 760 765
 GTT TCT ATT GTA AAA GCA TTA GAA CAT TTA CAT AGT AAG CTG TCT GTC
 CAA AGA TAA CAT TTT CGT AAT CTT GTA AAT GTA TCA TTC GAC AGA CAG
 Val Ser Ile Val Lys Ala Leu Glu His Leu His Ser Lys Leu Ser Val>
 770 775 780 785 790 795 800 805 810 815
 ATT CAC AGA GAC GTC AAG CCT TCT AAT GTA CTC ATC AAT GCT CTC GGT
 TAA GTG TCT CTG CAG TTC GGA AGA TTA CAT GAG TAG TTA CGA GAG CCA
 Ile His Arg Asp Val Lys Pro Ser Asn Val Leu Ile Asn Ala Leu Gly>
 820 825 830 835 840 845 850 855 860
 CAA GTG AAG ATG TGC GAT TTT GGA ATC AGT GGC TAC TTG GTG GAC TCT
 GTT CAC TTC TAC ACG CTA AAA CCT TAG TCA CCG ATG AAC CAC CTG AGA
 Gln Val Lys Met Cys Asp Phe Gly Ile Ser Gly Tyr Leu Val Asp Ser>
 865 870 875 880 885 890 895 900 905 910
 GTT GCT AAA ACA ATT GAT GCA GGT TGC AAA CCA TAC ATG GCC CCT GAA
 CAA CGA TTT TGT TAA CTA CGT CCA ACG TTT GGT ATG TAC CGG GGA CTT
 Val Ala Lys Thr Ile Asp Ala Gly Cys Lys Pro Tyr Met Ala Pro Glu>
 915 920 925 930 935 940 945 950 955 960
 AGA ATA AAC CCA GAG CTC AAC CAG AAG GGA TAC AGT GTG AAG TCT GAC
 TCT TAT TTG GGT CTC GAG TTG GTC TTC CCT ATG TCA CAC TTC AGA CTG
 Arg Ile Asn Pro Glu Leu Asn Gln Lys Gly Tyr Ser Val Lys Ser Asp>
 965 970 975 980 985 990 995 1000 1005
 ATT TGG AGT CTG GGC ATC ACG ATG ATT GAG TTG GCC ATC CTT CGA TTT
 TAA ACC TCA GAC CCG TAG TGC TAC TAA CTC AAC CGG TAG GAA GCT AAA
 Ile Trp Ser Leu Gly Ile Thr Met Ile Glu Leu Ala Ile Leu Arg Phe>
 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055
 CCC TAT GAT TCA TGG GGA ACT CCA TTT CAG CAG CTC AAA CAG GTG GTA
 GGG ATA CTA AGT ACC CCT TGA GGT AAA GTC GTC GAG TTT GTC CAC CAT
 Pro Tyr Asp Ser Trp Gly Thr Pro Phe Gln Gln Leu Lys Gln Val Val>

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FIG. 5 - CONT'D

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1060 1065 1070 1075 1080 1085 1090 1095 1100
*      *      *      *      *      *      *      *
GAG GAG CCA TCG CCA CAA CTC CCA GCA GAC AAG TTC TCT GCA GAG TTT
CTC CTC GGT AGC GGT GTT GAG GGT CGT CTG TTC AAG AGA CGT CTC AAA
Glu Glu Pro Ser Pro Gln Leu Pro Ala Asp Lys Phe Ser Ala Glu Phe>

1105 1110 1115 1120 1125 1130 1135 1140 1145 1150
*      *      *      *      *      *      *      *
GTT GAC TTT ACC TCA CAG TGC TTA AAG AAG AAT TCC AAA GAA CGG CCT
CAA CTG AAA TGG AGT GTC ACG AAT TTC TTC TTA AGG TTT CTT GCC GGA
Val Asp Phe Thr Ser Gln Cys Leu Lys Lys Asn Ser Lys Glu Arg Pro>

1155 1160 1165 1170 1175 1180 1185 1190 1195 1200
*      *      *      *      *      *      *      *
ACA TAC CCA GAG CTA ATG CAA CAT CCA TTT TTC ACC CTA CAT GAA TCC
TGT ATG GGT CTC GAT TAC GTT GTA GGT AAA AAG TGG GAT GTA CTT AGG
Thr Tyr Pro Glu Leu Met Gln His Pro Phe Phe Thr Leu His Glu Ser>

1205 1210 1215 1220 1225 1230 1235 1240 1245 1250
*      *      *      *      *      *      *      *
AAA GGA ACA GAT GTG GCA TCT TTT GTA AAA CTG ATT CTT GGA GAC TAAAA
TTT CCT TGT CTA CAC CGT AGA AAA CAT TTT GAC TAA GAA CCT CTG ATTTT
Lys Gly Thr Asp Val Ala Ser Phe Val Lys Leu Ile Leu Gly Asp> (SEQ ID NO:4)

1255 1260 1265 1270 1275 1280 1285 1290 1295 1300 1305 1310
*      *      *      *      *      *      *      *
AGCAGTGGAC TTAATCGGTT GACCCCTACTG TGGATTGGTG GGTTCGGGG TGAAGCAAGT
TCGTCACCTG AATTAGCCAA CTGGGATGAC ACCTAACCAC CCAAAGCCCC ACTTCGTTCA

1315 1320 1325 1330 1335 1340 1345 1350 1355 1360 1365 1370
*      *      *      *      *      *      *      *
TCACTACAGC ATCAATAGAA AGTCATCTTT GAGATAATTT AACCCTGCCT CTCAGAGGGT
AGTGATGTCG TAGTTATCTT TCAGTAGAAA CTCTATTAAA TTGGGACGGA GAGTCTCCCA

1375 1380 1385 1390 1395 1400 1405 1410 1415 1420 1425 1430
*      *      *      *      *      *      *      *
TTTCTCTCCC AATTTTCTTT TTAATCCCCC TCTTAAGGGG GCCTTGGAAT CTATAGTATA
AAAGAGAGGG TTAAAAGAAA AATGAGGGGG AGAATTCCCC CGGAACCTTA GATATCATAT

1435 1440 1445 1450 1455 1460 1465 1470 1475 1480 1485 1490
*      *      *      *      *      *      *      *
GAATGAACCTG TCTAGATGGA TGAATTATGA TAAAGGCTTA GGAATTCAAA AGGTGATTAA
CTTACTTGAC AGATCTACCT ACTTAATACT ATTTCOGAAT OCTGAAGTTT TCCACTAATT

1495 1500 1505 1510 1515 1520 1525 1530 1535 1540 1545 1550
*      *      *      *      *      *      *      *
ATATTTAATG ATGTGTCATA TGAGTCCTCA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
TATAAATTAC TACACAGTAT ACTCAGGAGT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT

1555 1560 1565 1570 1575 1580 1585 1590 1595 1600
*      *      *      *      *      *      *      *
AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AA (SEQ ID NO:3)
TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT TT

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FIG. 6

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      5    10    15    20    25    30    35    40    45    50    55
      *    *    *    *    *    *    *    *    *    *    *
CTAGGGTCCC CGGCGCCAGG CCACCCGGCC GTCAGCAGC ATG CAG GGT AAA CGC AAA
GATCCCAGGG GCCGCGGTCC GGTGGGCCGG CAGTCGTCG TAC GTC CCA TTT GCG TTT
                               Met Gln Gly Lys Arg Lys>

      60    65    70    75    80    85    90    95    100   105
      *    *    *    *    *    *    *    *    *    *    *
GCA CTG AAG TTG AAT TTT GCA AAT CCA CCT TTC AAA TCT ACA GCA AGG
CGT GAC TTC AAC TTA AAA CGT TTA GGT GGA AAG TTT AGA TGT CGT TCC
Ala Leu Lys Leu Asn Phe Ala Asn Pro Pro Phe Lys Ser Thr Ala Arg>

      110   115   120   125   130   135   140   145   150
      *    *    *    *    *    *    *    *    *
TTT ACT CTG AAT CCC AAT CCT ACA GGA GTT CAA AAC CCA CAC ATA GAG
AAA TGA GAC TTA GGG TTA GGA TGT CCT CAA GTT TTG GGT GTG TAT CTC
Phe Thr Leu Asn Pro Asn Pro Thr Gly Val Gln Asn Pro His Ile Glu>

      155   160   165   170   175   180   185   190   195   200
      *    *    *    *    *    *    *    *    *    *
AGA CTG AGA ACA CAC AGC ATT GAG TCA TCA GGA AAA CTG AAG ATC TCC
TCT GAC TCT TGT GTG TCG TAA CTC AGT AGT CCT TTT GAC TTC TAG AGG
Arg Leu Arg Thr His Ser Ile Glu Ser Ser Gly Lys Leu Lys Ile Ser>

      205   210   215   220   225   230   235   240   245
      *    *    *    *    *    *    *    *    *
CCT GAA CAA CAC TGG GAT TTC ACT GCA GAG GAC TTG AAA GAC CTT GGA
GGA CTT GTT GTG ACC CTA AAG TGA CGT CTC CTG AAC TTT CTG GAA CTT
Pro Glu Gln His Trp Asp Phe Thr Ala Glu Asp Leu Lys Asp Leu Gly>

      250   255   260   265   270   275   280   285   290   295
      *    *    *    *    *    *    *    *    *    *
GAA ATT GGA CGA GGA GCT TAT GGT TCT GTC AAC AAA ATG GTC CAC AAA
CTT TAA CCT GCT CCT CGA ATA CCA AGA CAG TTG TTT TAC CAG GTG TTT
Glu Ile Gly Arg Gly Ala Tyr Gly Ser Val Asn Lys Met Val His Lys>

      300   305   310   315   320   325   330   335   340   345
      *    *    *    *    *    *    *    *    *    *
CCA AGT GGG CAA ATA ATG GCA GTT AAA AGA ATT CGG TCA ACA GTG GAT
GGT TCA CCC GTT TAT TAC CGT CAA TTT TCT TAA GCC AGT TGT CAC CTA
Pro Ser Gly Gln Ile Met Ala Val Lys Arg Ile Arg Ser Thr Val Asp>

      350   355   360   365   370   375   380   385   390
      *    *    *    *    *    *    *    *    *
GAA AAA GAA CAA AAA CAA CTT CTT ATG GAT TTG GAT GTA GTA ATG CGG
CTT TTT CTT GTT TTT GTT GAA GAA TAC CTA AAC CTA CAT CAT TAC GCC
Glu Lys Glu Gln Lys Gln Leu Leu Met Asp Leu Asp Val Val Met Arg>

      395   400   405   410   415   420   425   430   435   440
      *    *    *    *    *    *    *    *    *    *
AGT AGT GAT TGC CCA TAC ATT GTT CAG TTT TAT GGT GCA CTC TTC AGA
TCA TCA CTA ACG GGT ATG TAA CAA GTC AAA ATA CCA CGT GAG AAG TCT
Ser Ser Asp Cys Pro Tyr Ile Val Gln Phe Tyr Gly Ala Leu Phe Arg>

      445   450   455   460   465   470   475   480   485
      *    *    *    *    *    *    *    *    *
GAG GGT GAC TGT TGG ATC TGT ATG GAA CTC ATG TCT ACC TCG TTT GAT
CTC CCA CTG ACA ACC TAG ACA TAC CTT GAG TAC AGA TGG AGC AAA CTA
Glu Gly Asp Cys Trp Ile Cys Met Glu Leu Met Ser Thr Ser Phe Asp>

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FIG. 6 - CONT'D

490 495 500 505 510 515 520 525 530 535
 * * * * * * * * *
 AAG TTT TAC AAA TAT GTA TAT AGT GTA TTA GAT GAT GTT ATT CCA GAA
 TTC AAA ATG TTT ATA CAT ATA TCA CAT AAT CTA CTA CAA TAA GGT CTT
 Lys Phe Tyr Lys Tyr Val Tyr Ser Val Leu Asp Asp Val Ile Pro Glu>
 540 545 550 555 560 565 570 575 580 585
 * * * * * * * * *
 GAA ATT TTA GGC AAA ATC ACT TTA GCA ACT GTG AAA GCA CTA AAC CAC
 CTT TAA AAT CCG TTT TAG TGA AAT CGT TGA CAC TTT CGT GAT TTG GTG
 Glu Ile Leu Gly Lys Ile Thr Leu Ala Thr Val Lys Ala Leu Asn His>
 590 595 600 605 610 615 620 625 630
 * * * * * * * * *
 TTA AAA GAA AAC TTG AAA ATT ATT CAC AGA GAT ATC AAA CCT TCC AAT
 AAT TTT CTT TTG AAC TTT TAA TAA GTG TCT CTA TAG TTT GGA AGG TTA
 Leu Lys Glu Asn Leu Lys Ile Ile His Arg Asp Ile Lys Pro Ser Asn>
 635 640 645 650 655 660 665 670 675 680
 * * * * * * * * *
 ATT CTT CTG GAC AGA AGT GGA AAT ATT AAG CTC TGT GAC TTC GGC ATC
 TAA GAA GAC CTG TCT TCA CCT TTA TAA TTC GAG ACA CTG AAG CCG TAG
 Ile Leu Leu Asp Arg Ser Gly Asn Ile Lys Leu Cys Asp Phe Gly Ile>
 685 690 695 700 705 710 715 720 725
 * * * * * * * * *
 AGT GGA CAG CTT GTG GAC TCT ATT GCC AAG ACA AGA GAT GCT GGC TGT
 TCA CCT GTC GAA CAC CTG AGA TAA CCG TTC TGT TCT CTA CGA CCG ACA
 Ser Gly Gln Leu Val Asp Ser Ile Ala Lys Thr Arg Asp Ala Gly Cys>
 730 735 740 745 750 755 760 765 770 775
 * * * * * * * * *
 AGG CCA TAC ATG GCA CCT GAA AGA ATA GAC CCA AGC GCA TCA CGA CAA
 TCC GGT ATG TAC CGT GGA CTT TCT TAT CTG GGT TCG CGT AGT GCT GTT
 Arg Pro Tyr Met Ala Pro Glu Arg Ile Asp Pro Ser Ala Ser Arg Gln>
 780 785 790 795 800 805 810 815 820 825
 * * * * * * * * *
 GGA TAT GAT GTC CGC TCT GAT GTC TGG AGT TTG GGG ATC ACA TTG TAT
 CCT ATA CTA CAG GCG AGA CTA CAG ACC TCA AAC CCC TAG TGT AAC ATA
 Gly Tyr Asp Val Arg Ser Asp Val Trp Ser Leu Gly Ile Thr Leu Tyr>
 830 835 840 845 850 855 860 865 870
 * * * * * * * * *
 GAG TTG GCC ACA GGC CGA TTT CCT TAT CCA AAG TGG AAT AGT GTA TTT
 CTC AAC CGG TGT CCG GCT AAA GGA ATA GGT TTC ACC TTA TCA CAT AAA
 Glu Leu Ala Thr Gly Arg Phe Pro Tyr Pro Lys Trp Asn Ser Val Phe>
 875 880 885 890 895 900 905 910 915 920
 * * * * * * * * *
 GAT CAA CTA ACA CAA GTC GTG AAA GGA GAT CCT CCG CAG CTG AGT AAT
 CTA GTT GAT TGT GTT CAG CAC TTT CCT CTA GGA GGC GTC GAC TCA TTA
 Asp Gln Leu Thr Gln Val Val Lys Gly Asp Pro Pro Gln Leu Ser Asn>
 925 930 935 940 945 950 955 960 965
 * * * * * * * * *
 TCT GAG GAA AGG GAA TTC TCC CCG AGT TTC ATC AAC TTT GTC AAC TTG
 AGA CTC CTT TCC CTT AAG AGG GGC TCA AAG TAG TTG AAA CAG TTG AAC
 Ser Glu Arg Glu Phe Ser Pro Ser Phe Ile Asn Phe Val Asn Leu>
 970 975 980 985 990 995 1000 1005 1010 1015
 * * * * * * * * *

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FIG. 6 - CONT'D

TGC CTT ACG AAG GAT GAA TCC AAA AGG CCA AAG TAT AAA GAG CTT CTG
 ACG GAA TGC TTC CTA CTT AGG TTT TCC GGT TTC ATA TTT CTC GAA GAC
 Cys Leu Thr Lys Asp Glu Ser Lys Arg Pro Lys Tyr Lys Glu Leu Leu>

1020 1025 1030 1035 1040 1045 1050 1055 1060 1065
 * * * * *
 AAA CAT CCC TTT ATT TTG ATG TAT GAA GAA CGT GCC GTT GAG GTC GCA
 TTT GTA GGG AAA TAA AAC TAC ATA CTT CTT GCA CGG CAA CTC CAG CGT
 Lys His Pro Phe Ile Leu Met Tyr Glu Glu Arg Ala Val Glu Val Ala>

1070 1075 1080 1085 1090 1095 1100 1105 1110
 * * * * *
 TGC TAT GTT TGT AAA ATC CTG GAT CAA ATG CCA GCT ACT CCC AGC TCT
 ACG ATA CAA ACA TTT TAG GAC CTA GTT TAC GGT CGA TGA GGG TCG AGA
 Cys Tyr Val Cys Lys Ile Leu Asp Gln Met Pro Ala Thr Pro Ser Ser>

1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170
 * * * * *
 CCC ATG TAT GTC GAT TG ATATCGYTGC TACATCAGAC TCTAGAAAAA AGGGCTGAGA
 GGG TAC ATA CAG CTA AC TATAGCRACG ATGTAGTCTG AGATCTTTTT TCCCAGACTCT
 Pro Met Tyr Val Asp> (SEQ ID NO:6)

1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 1225 1230
 * * * * *
 GGAAGCAAGA CGTAAAGAAT TTTCATCCCG TATCACAGTG TTTTATTGTC TCGCCAGAC
 CCTTCGTTCT GCATTTCTTA AAAGTAGGGC ATAGTGTAC AAAAAAATACG AGCGGGTCTG

1235 1240 1245 1250 1255 1260 1265 1270 1275 1280 1285 1290
 * * * * *
 ACCATGTGCA ATAAGATTGG TGTTCTGTTTC CATCATGTCT GTATACTCCT GTCACCTAGA
 TGGTACACGT TATTCTAACC ACAAGCAAAG GTAGTACAGA CATATGAGGA CAGTGGATCT

1295 1300 1305 1310 1315 1320 1325 1330 1335 1340 1345 1350
 * * * * *
 ACGTGCATCC TTGTAATACC TGATTGATCA CACAGTGTGA GTGCTGGTCA GAGAGACCTC
 TGCACGTAGG AACATTATGG ACTAAGTAGT GTGTACAAAT CACGACCACT CTCTCTGGAG

1355 1360 1365 1370 1375 1380 1385 1390 1395 1400 1405 1410
 * * * * *
 ATCCTGCTCT TTTGTGATGA ACATATTCAT GAAATGTGGA AGTCAGTACG ATCAAGTTGT
 TAGGACGAGA AAACACTACT TGTATAAGTA CTTTACACCT TCAGTCATGC TAGTTCAACA

1415 1420 1425 1430 1435 1440 1445 1450 1455 1460 1465 1470
 * * * * *
 TGA CTGTGAT TAGATCAGAT CTTAAATTCA TTCTAGACT CAAAACCTGG AGATGCAGCT
 ACTGACACTA ATCTAGTGTA GAATTAAAGT AAAGATCTGA GTTTTGGACC TCTACGTCGA

1475 1480 1485 1490 1495 1500 1505 1510 1515 1520 1525 1530
 * * * * *
 ACTGGAATGG TGTTTTGTGA GACTTCCAAA TCCTGGAAGG ACACAGTGAT GAATGTACTA
 TGACCTTACC ACAAACAGT CTGAAGGTTT AGGACCTTCC TGTGTCACCTA CTTACATGAT

1535 1540 1545 1550 1555 1560 1565 1570 1575 1580 1585 1590
 * * * * *
 TATCTGAACA TAGAACTCG GGCTTGAGTG AGAAGAGCTT GCACAGCCAA CGAGACACAT
 ATAGACTTGT ATCTTTGAGC CCGAACTCAC TCTTCTCGAA CGTGTGGTT GCTCTGTGTA

1595 1600 1605 1610 1615 1620 1625 1630 1635 1640 1645 1650
 * * * * *
 TGCTTCTCG AGCTGGGAGA CAAAGGAGGA ATTTACTTTC TTCACCAAGT GCAATAGATT
 ACGGAAGACC TCGACCTCT GTTCTCTCT TAAATGAAAG AAGTGGTTCA CGTTATCTAA

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FIG. 6 - CONT'D

1655	1660	1665	1670	1675	1680	1685	1690	1695	1700	1705	1710
ACTGATGTGA	TATTCTGTTG	CTTTACAGTT	ACAGTTGATG	TTTGGGGATC	GATGTGCTCA						
TGACTACACT	ATAAGACAAC	GAAATGTCAA	TGTCAACTAC	AAACCCCTAG	CTACACGAGT						
1715	1720	1725	1730	1735	1740	1745	1750	1755	1760	1765	1770
GCCAAATTTT	CTGTTTGAAA	TATCATGTTA	AATTAGAATG	AATTTATCTT	TACCAAAAAC						
CGGTTTAAAG	GACAAACTTT	ATAGTACAAT	TTAATCTTAC	TTAAATAGAA	ATGGTMTTGT						
1775	1780	1785	1790	1795	1800	1805	1810	1815	1820	1825	1830
CATGTTCGGT	TCAAAGAGGT	GAACATTAAA	ATATAGAGAC	AGGACAGAAT	GTGTTCTTTT						
GTACAACGCA	AGTTTCTCCA	CTTGTAATTT	TATATCTCTG	TCCTGTCTTA	CACAAGAAAA						
1835	1840	1845	1850	1855	1860	1865	1870	1875	1880	1885	1890
CTCCTCTACC	AGTCCTATTT	TTCAATGGGA	AGACTCAGGA	GTCTGCCACT	TGTCAAAGAA						
GAGGAGATGG	TCAGGATAAA	AAGTTACCCT	TCTGAGTCCT	CAGACGGTGA	ACAGTTTCTT						
1895	1900	1905	1910	1915	1920	1925	1930	1935	1940	1945	1950
GGTGCTGATC	CTAAGAATTT	TTCATTCTCA	GAATTCGGTG	TGCTGCCAAC	TTGATGTTCC						
CCACGACTAG	GATTCTTAAA	AAGTAAGAGT	CTTAAGCCAC	ACGACGGTTG	AACTACAAGG						
1955	1960	1965	1970	1975	1980	1985	1990	1995	2000	2005	2010
ACCTGCCACA	AACCACCAGG	ACTGAAAGAA	GAAAACAGTA	CAGAAGGCAA	AGTTTACAGA						
TGGACGGTGT	TTGGTGGTCC	TGACTTTCTT	CTTTTGTCAT	GTCTTCCGTT	TCAAATGTCT						
2015	2020	2025	2030	2035	2040	2045	2050	2055	2060	2065	2070
TGTTTTTAAT	TCTAGTATTT	TATCTGGAAC	AACTTGTAGC	AGCTATATAT	TTCCCCCTGG						
ACAAAAATTA	AGATCATAAA	ATAGACCTTG	TTGAACATCG	TCGATATATA	AAGGGGAACC						
2075	2080	2085	2090	2095	2100	2105	2110	2115	2120	2125	2130
TCCCAAGCCT	GATACTTTAG	CCATCATAAC	TCACTAACAG	GGAGAAGTAG	CTAGTAGCAA						
AGGGTTCCGA	CTATGAAATC	GGTAGTATTG	AGTGATTGTC	CCTCTTCATC	GATCATCGTT						
2135	2140	2145	2150	2155	2160	2165	2170	2175	2180	2185	2190
TGTGCCTTGA	TTGATTAGAT	AAAGATTTCT	AGTAGGCAGC	AAAAGACCAA	ATCTCAGTTG						
ACACGGAACT	AACTAATCTA	TTTCTAAAGA	TCATCCGTCG	TTTTCTGGTT	TAGAGTCAAC						
2195	2200	2205	2210	2215	2220	2225	2230	2235	2240	2245	2250
TTTGCTTCTT	GCCATCACTG	GTCCAGGTCT	TCAGTTTCCG	AATCTCTTTC	CCTTCCCCCTG						
AAACGAAGAA	CGGTAGTGAC	CAGGTCCAGA	AGTCAAAGGC	TTAGAGAAAG	GGAAGGGGAC						
2255	2260	2265	2270	2275	2280	2285	2290	2295	2300	2305	2310
TGGTCTATTG	TCGCTATGTG	ACTTGCGCTT	AATCCAATAT	TTTGCCTTT	TTCTATATCA						
ACCAGATAAC	AGCGATACAC	TGAACCGGAA	TTAGGTTATA	AAACGGAAAA	AAGATATAGT						
2315	2320	2325	2330	2335	2340	2345	2350	2355	2360	2365	2370
AAAAACCTTT	ACAGTTAGCA	GGGATGTTCC	TTACCGAGGA	TTTTTAACCC	CCAATCTCTC						
TTTTTGGAAA	TGTCAATCGT	CCCTACAAGG	AATGGCTCCT	AAAAATTGGG	GGTTAGAGAG						
2375	2380	2385	2390	2395	2400	2405	2410	2415	2420	2425	2430

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FIG. 6 - CONT'D

ATAATCGCTA	GTGTTTAAAA	GGCTAAGAAT	AGTGGGGCCC	AACCGATGTG	GTAGGTGATA
TATTAGCGAT	CACAAATTTT	CCGATTCTTA	TCACCCCGGG	TTGGCTACAC	CATCCACTAT
2435 2440	2445 2450	2455 2460	2465 2470	2475 2480	2485 2490
AAGAGGCATC	TTTCTAGAG	ACACATTGGA	CCAGATGAGG	ATCCGAAACG	GCAGCCTTTA
TTCTCCGTAG	AAAAGATCTC	TGTGTAACCT	GGTCTACTCC	TAGGCTTTGC	CGTCGGAAAT
2495 2500	2505 2510	2515 2520	2525 2530	2535 2540	2545 2550
CGTTCATCAC	CTGCTAGAAC	CTCTCGTAGT	CCATCACCAT	TTCTTGGCAT	TGGAATTCTA
GCAAGTAGTG	GACGATCTTG	GAGAGCATCA	GGTAGTGGTA	AAGAACCGTA	ACCTTAAGAT
2555 2560	2565 2570	2575 2580	2585 2590	2595 2600	2605 2610
CTGGAAAAAA	ATACAAAAAG	CAAAACAAAA	CCCTCAGCAC	TGTTACAAGA	GGCCATTTTA
GACCTTTTTT	TATGTTTTTC	GTTTTGTTTT	GGGAGTCGTG	ACAATGTTCT	CCGGTAAATT
2615 2620	2625 2630	2635 2640	2645 2650	2655 2660	2665 2670
GTATCTGTG	CTTCTTCACT	TACCCATTAG	CCAGGTTCTC	ATTAGGTTTT	GCTTGGGCCT
CATAGAACAC	GAAGAAGTGA	ATGGGTAATC	GGTCCAAGAG	TAATCCAAAA	CGAACCCGGA
2675 2680	2685 2690	2695 2700	2705 2710	2715 2720	2725 2730
CCCTGGCACT	GAACCTTAGG	CTTTGTATGA	CAGTGAAGCA	GCACTGTGAG	TGGTCAAGC
GGGACCGTGA	CTTGGAATCC	GAAACATACT	GTCACTTCGT	CGTGACACTC	ACCAAGTTCC
2735 2740	2745 2750	2755 2760	2765 2770	2775 2780	2785 2790
ACACTGGAAT	ATAAAACAGT	CATGGCCTGA	GATGCAGGTG	ATGCCATTAC	AGAACCAAT
TGTGACCTTA	TATTTTGTCA	GTACCGGACT	CTACGTCCAC	TACGGTAATG	TCTTGGTTTA
2795 2800	2805 2810	2815 2820	2825 2830	2835 2840	2845 2850
CGTGGCAAGT	ATTGCTGTGT	CTCCCTCAG	AGTGACAGTC	ATAAATACTG	TCAAACAATA
GCACCGTGCA	TAACGACACA	GAGGAGAGTC	TCACTGTCAG	TATTTATGAC	AGTTTGTAT
2855 2860	2865 2870	2875 2880	2885 2890	2895 2900	2905 2910
AAGGGAGAAT	GGTGCTGTTT	AAAGTCACAT	CCCTGTAAAT	TGCAGAAATC	AAAAGTGATT
TTCCCTCTTA	CCACGACAAA	TTTCAGTGT	GGGACATTTA	ACGTCCTAAG	TTTTCACTAA
2915 2920	2925 2930	2935 2940	2945 2950	2955 2960	2965 2970
ATCTCTTTGA	TCTACTTGCC	TCATTTCCCT	ATCTTCTCCC	CCACGGTATC	CTAAACTTTA
TAGAGAAACT	AGATGAACGG	AGTAAAGGGA	TAGAAGAGGG	GGTGCCATAG	GATTTGAAAT
2975 2980	2985 2990	2995 3000	3005 3010	3015 3020	3025 3030
GACTTCCAC	TGTTCTGAAA	GGAGACATTG	CTCTATGTCT	GCCTTCGACC	ACAGCAAGCC
CTGAAGGGTG	ACAAGACTTT	CCTCTGTAAC	GAGATACAGA	CGGAAGCTGG	TGTCGTTCCG
3035 3040	3045 3050	3055 3060	3065 3070	3075 3080	3085 3090
ATCATCCTCC	ATTGCTCCCG	GGGACTCAAG	AGGAATCTGT	TTCTCTGCTG	TCAACTTCCC
TAGTAGGAGG	TAACGAGGGC	CCCTGAGTTC	TCCTTAGACA	AAGAGACGAC	AGTTGAAGGG
3095 3100	3105 3110	3115 3120	3125 3130	3135 3140	3145 3150
ATCTGGCTCA	GCATAGGGTC	ACTTTGCCAT	TATGCAAATG	GAGATAAAAG	CAATTCTGGC
TAGACCGAGT	CGTATCCCG	TGAAACGGTA	ATACGTTTAC	CTCTATTTTC	GTTAAGACCG

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FIG. 6 - CONT'D

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3155 3160 3165 3170 3175 3180 3185 3190 3195 3200 3205 3210
      *      *      *      *      *      *      *      *
TGTCAGGAG CTAATCTGAC CGTTCCTATTG TGTGGATGAC CACATAAGAA GGCAATTTTA
ACAGGTCCTC GATTAGACTG GCAAGATAAC ACACCTACTG GTGTATTCTT CCGTTAAAAT

3215 3220 3225 3230 3235 3240 3245 3250 3255 3260 3265 3270
      *      *      *      *      *      *      *      *
GTGTATTAAT CATAGATTAT TATAAACTAT AAACCTTAAGG GCAAGGAGTT TATTACAATG
CACATAATTA GTATCTAATA ATATTGATA TTGAATTCC CGTTCCTCAA ATAATGTTAC

3275 3280 3285 3290 3295 3300 3305 3310 3315 3320 3325 3330
      *      *      *      *      *      *      *      *
TATCTTTATT AAAACAAAAG GGTGTATAGT GTTCACAAAC TGTGAAAATA GTGTAAGAAC
ATAGAAATAA TTTTGTTTTC CCACATATCA CAAGTGTTTG AACTTTTAT CACATTCTTG

3335 3340 3345 3350 3355 3360 3365 3370 3375 3380 3385 3390
      *      *      *      *      *      *      *      *
TGTACATTGT GAGCTCTGGT TATTTTCTCT TTGTACCATA GAAAAATGTA TAAAAATTAT
ACATGTAACA CTCGAGACCA ATAAAAAGAG AACATGGTAT CTTTTTACAT ATTTTAAATA

3395 3400 3405 3410 3415 3420 3425 3430 3435 3440 3445 3450
      *      *      *      *      *      *      *      *
CAAAAAGCTA ATGTGCAGGG ATATTGCCTT ATTTGTCTGT AAAAAATGGA GCTCAGTAAC
GTTTTTCGAT TACACGTCCC TATAACGGAA TAAACAGACA TTTTTTACCT CGAGTCATTG

3455 3460 3465 3470 3475 3480 3485 3490 3495
      *      *      *      *      *      *      *
ATAACTGCTT CTTGGAGCTT TGAATATTT TATCCTGTAT TCTTGTTT (SEQ ID NO:5)
TATTGACGAA GAACCTCGAA ACCTTATAAA ATAGGACATA AGAACAAA
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FIG. 7

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      5      10      15      20      25      30      35      40      45      50
      *      *      *      *      *      *      *      *      *      *
CAACA ATG GCG GCT CCG AGC CCG AGC GGT GGC GGC GGC AGC GGC ACC CCC
GTTGT TAC CGC CGA GGC TCG GGC TCG CCA CCG CCG CCG TCG CCG TGG GGG
Met Ala Ala Pro Ser Pro Ser Gly Gly Gly Ser Gly Thr Pro>

      55      60      65      70      75      80      85      90      95
      *      *      *      *      *      *      *      *      *
GGC CCC GTA GGG TCC CCG GCG CCA GGC CAC CCG GCC GTC AGC AGC ATG
CCG GGG CAT CCC AGG GGC CGC GGT CCG GTG GGC CGG CAG TCG TCG TAC
Gly Pro Val Gly Ser Pro Ala Pro Gly His Pro Ala Val Ser Ser Met>

100    105    110    115    120    125    130    135    140    145
*      *      *      *      *      *      *      *      *      *
CAG GGT AAA CGC AAA GCA CTG AAG TTG AAT TTT GCA AAT CCA CCT TTC
GTC CCA TTT GCG TTT CGT GAC TTC AAC TTA AAA CGT TTA GGT GGA AAG
Gln Gly Lys Arg Lys Ala Leu Lys Leu Asn Phe Ala Asn Pro Pro Phe>

      150     155     160     165     170     175     180     185     190
      *      *      *      *      *      *      *      *      *
AAA TCT ACA GCA AGG TTT ACT CTG AAT CCC AAT CCT ACA GGA GTT CAA
TTT AGA TGT CGT TCC AAA TGA GAC TTA GGG TTA GGA TGT CCT CAA GTT
Lys Ser Thr Ala Arg Phe Thr Leu Asn Pro Asn Pro Thr Gly Val Gln>

195    200    205    210    215    220    225    230    235    240
*      *      *      *      *      *      *      *      *      *
AAC CCA CAC ATA GAG AGA CTG AGA ACA CAC AGC ATT GAG TCA TCA GGA
TTG GGT GTG TAT CTC TCT GAC TCT TGT GTG TCG TAA CTC AGT AGT CCT
Asn Pro His Ile Glu Arg Leu Arg Thr His Ser Ile Glu Ser Ser Gly>

      245     250     255     260     265     270     275     280     285     290
      *      *      *      *      *      *      *      *      *      *
AAA CTG AAG ATC TCC CCT GAA CAA CAC TGG GAT TTC ACT GCA GAG GAC
TTT GAC TTC TAG AGG GGA CTT GTT GTG ACC CTA AAG TGA CGT CTC CTG
Lys Leu Lys Ile Ser Pro Glu Gln His Trp Asp Phe Thr Ala Glu Asp>

      295     300     305     310     315     320     325     330     335
      *      *      *      *      *      *      *      *      *
TTG AAA GAC CTT GGA GAA ATT GGA CGA GGA GCT TAT GGT TCT GTC AAC
AAC TTT CTG GAA CCT CTT TAA CCT GCT CCT CGA ATA CCA AGA CAG TTG
Leu Lys Asp Leu Gly Glu Ile Gly Arg Gly Ala Tyr Gly Ser Val Asn>

340    345    350    355    360    365    370    375    380    385
*      *      *      *      *      *      *      *      *      *
AAA ATG GTC CAC AAA CCA AGT GGG CAA ATA ATG GCA GTT AAA AGA ATT
TTT TAC CAG GTG TTT GGT TCA CCC GTT TAT TAC CGT CAA TTT TCT TAA
Lys Met Val His Lys Pro Ser Gly Gln Ile Met Ala Val Lys Arg Ile>

      390     395     400     405     410     415     420     425     430
      *      *      *      *      *      *      *      *      *
CGG TCA ACA GTG GAT GAA AAA GAA CAA AAA CAA CTT CTT ATG GAT TTG
GCC AGT TGT CAC CTA CTT TTT CTT GTT TTT GTT GAA GAA TAC CTA AAC
Arg Ser Thr Val Asp Glu Lys Glu Gln Lys Gln Leu Leu Met Asp Leu>

435    440    445    450    455    460    465    470    475    480
*      *      *      *      *      *      *      *      *      *
GAT GTA GTA ATG CGG AGT AGT GAT TGC CCA TAC ATT GTT CAG TTT TAT
CTA CAT CAT TAC GCC TCA TCA CTA ACG GGT ATG TAA CAA GTC AAA ATA
Asp Val Val Met Arg Ser Ser Asp Cys Pro Tyr Il Val Gln Phe Tyr>

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FIG. 7 - CONT'D

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485      490      495      500      505      510      515      520      525      530
      *      *      *      *      *      *      *      *      *
GGT GCA CTC TTC AGA GAG GGT GAC TGT TGG ATC TGT ATG GAA CTC ATG
CCA CGT GAG AAG TCT CTC CCA CTG ACA ACC TAG ACA TAC CTT GAG TAC
Gly Ala Leu Phe Arg Glu Gly Asp Cys Trp Ile Cys Met Glu Leu Met>

      535      540      545      550      555      560      565      570      575
      *      *      *      *      *      *      *      *      *
TCT ACC TCG TTT GAT AAG TTT TAC AAA TAT GTA TAT AGT GTA TTA GAT
AGA TGG AGC AAA CTA TTC AAA ATG TTT ATA CTT ATA TCA CAT AAT CTA
Ser Thr Ser Phe Asp Lys Phe Tyr Lys Tyr Val Tyr Ser Val Leu Asp>

580      585      590      595      600      605      610      615      620      625
      *      *      *      *      *      *      *      *      *
GAT GTT ATT CCA GAA GAA ATT TTA GGC AAA ATC ACT TTA GCA ACT GTG
CTA CAA TAA GGT CTT CTT TAA AAT CCG TTT TAG TGA AAT CGT TGA CAC
Asp Val Ile Pro Glu Glu Ile Leu Gly Lys Ile Thr Leu Ala Thr Val>

      630      635      640      645      650      655      660      665      670
      *      *      *      *      *      *      *      *      *
AAA GCA CTA AAC CAC TTA AAA GAA AAC TTG AAA ATT ATT CAC AGA GAT
TTT CGT GAT TTG GTG AAT TTT CTT TTG AAC TTT TAA TAA GTG TCT CTA
Lys Ala Leu Asn His Leu Lys Glu Asn Leu Lys Ile Ile His Arg Asp>

675      680      685      690      695      700      705      710      715      720
      *      *      *      *      *      *      *      *      *
ATC AAA CCT TCC AAT ATT CTT CTG GAC AGA AGT GGA AAT ATT AAG CTC
TAG TTT GGA AGG TTA TAA GAA GAC CTG TCT TCA CCT TTA TAA TTC GAG
Ile Lys Pro Ser Asn Ile Leu Leu Asp Arg Ser Gly Asn Ile Lys Leu>

725      730      735      740      745      750      755      760      765      770
      *      *      *      *      *      *      *      *      *
TGT GAC TTC GGC ATC AGT GGA CAG CTT GTG GAC TCT ATT GCC AAG ACA
ACA CTG AAG CCG TAG TCA CCT GTC GAA CAC CTG AGA TAA CGG TTC TGT
Cys Asp Phe Gly Ile Ser Gly Gln Leu Val Asp Ser Ile Ala Lys Thr>

      775      780      785      790      795      800      805      810      815
      *      *      *      *      *      *      *      *      *
AGA GAT GCT GGC TGT AGG CCA TAC ATG GCA CCT GAA AGA ATA GAC CCA
TCT CTA CGA CCG ACA TCC GGT ATG TAC CGT GGA CTT TCT TAT CTG GGT
Arg Asp Ala Gly Cys Arg Pro Tyr Met Ala Pro Glu Arg Ile Asp Pro>

820      825      830      835      840      845      850      855      860      865
      *      *      *      *      *      *      *      *      *
AGC GCA TCA CGA CAA GGA TAT GAT GTC CGC TCT GAT GTC TGG AGT TTG
TCG CGT AGT GCT GTT CCT ATA CTA CAG GCG AGA CTA CAG ACC TCA AAC
Ser Ala Ser Arg Gln Gly Tyr Asp Val Arg Ser Asp Val Trp Ser Leu>

      870      875      880      885      890      895      900      905      910
      *      *      *      *      *      *      *      *      *
GGG ATC ACA TTG TAT GAG TTG GCC ACA GGC CGA TTT CCT TAT CCA AAG
CCC TAG TGT AAC ATA CTC AAC CGG TGT CCG GCT AAA GGA ATA GGT TTC
Gly Ile Thr Leu Tyr Glu Leu Ala Thr Gly Arg Phe Pro Tyr Pro Lys>

915      920      925      930      935      940      945      950      955      960
      *      *      *      *      *      *      *      *      *
TGG AAT AGT GTA TTT GAT CAA CTA ACA CAA GTC GTG AAA GGA GAT CCT
ACC TTA TCA CAT AAA CTA GTT GAT TGT GTT CAG CAC TTT CCT CTA GGA
Trp Asn Ser Val Phe Asp Gln Leu Thr Gln Val Val Lys Gly Asp Pro>

965      970      975      980      985      990      995      1000      1005      1010
      *      *      *      *      *      *      *      *      *

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FIG. 7 - CONT'D

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CCG CAG CTG AGT AAT TCT GAG GAA AGG GAA TTC TCC CCG AGT TTC ATC
GGC GTC GAC TCA TTA AGA CTC CTT TCC CTT AAG AGG GGC TCA AAG TAG
Pro Gln Leu Ser Asn Ser Glu Glu Arg Glu Phe Ser Pro Ser Phe Ile>

1015 1020 1025 1030 1035 1040 1045 1050 1055
* * * * *
AAC TTT GTC AAC TTG TGC CTT ACG AAG GAT GAA TCC AAA AGG CCA AAG
TTG AAA CAG TTG AAC ACG GAA TGC TTC CTA CTT AGG TTT TCC GGT TTC
Asn Phe Val Asn Leu Cys Leu Thr Lys Asp Glu Ser Lys Arg Pro Lys>

1060 1065 1070 1075 1080 1085 1090 1095 1100 1105
* * * * *
TAT AAA GAG CTT CTG AAA CAT CCC TTT ATT TTG ATG TAT GAA GAA CGT
ATA TTT CTC GAA GAC TTT GTA GGG AAA TAA AAC TAC ATA CTT CTT GCA
Tyr Lys Glu Leu Leu Lys His Pro Phe Ile Leu Met Tyr Glu Glu Arg>

1110 1115 1120 1125 1130 1135 1140 1145 1150
* * * * *
GCC GTT GAG GTC GCA TGC TAT GTT TGT AAA ATC CTG GAT CAA ATG CCA
CGG CAA CTC CAG CGT ACG ATA CAA ACA TTT TAG GAC CTA GTT TAC GGT
Ala Val Glu Val Ala Cys Tyr Val Cys Lys Ile Leu Asp Gln Met Pro>

1155 1160 1165 1170 1175 1180 1185 1190 1195 1200
* * * * *
GCT ACT CCC AGC TCT CCC ATG TAT GTC GAT TGATAT CGYTGCTACA
CGA TGA GGG TCG AGA GGG TAC ATA CAG CTA ACTATA GCRACGATGT
Ala Thr Pro Ser Ser Pro Met Tyr Val Asp> (SEQ ID NO:8)

1205 1210 1215 1220 1225 1230 1235 1240 1245 1250 1255 1260
* * * * *
TCAGACTCTA GAAAAAAGGG CTGAGAGGAA GCAAGACGTA AAGAATTTTC ATCCCGTATC
AGTCTGAGAT CTTTTTCCG GACTCTCCTT CGTTCTGCAT TTCTTAAAAG TAGGGCATAG

1265 1270 1275 1280 1285 1290 1295 1300 1305 1310 1315 1320
* * * * *
ACAGTGT TTT TATTGCTCGC CCAGACACCA TGTGCAATAA GATTGGTGT CGTTTCCATC
TGTCACAAAA ATAACGAGCG GGTCTGTGGT ACACGTTATT CTAACCACAA GCAAAGGTAG

1325 1330 1335 1340 1345 1350 1355 1360 1365 1370 1375 1380
* * * * *
ATGTCTGTAT ACTCTGTGCA CCTAGAACGT GCATCCTTGT AATACCTGAT TGATCACACA
TACAGACATA TGAGGACAGT GGATCTTGCA CGTAGGAACA TTATGGACTA ACTAGTGTGT

1385 1390 1395 1400 1405 1410 1415 1420 1425 1430 1435 1440
* * * * *
GTGTTAGTGC TGGTCAGAGA GACCTCATCC TGCTCTTTTG TGATGAACAT ATTTCATGAAA
CACAAATCAG ACCAGTCTCT CTGGAGTAGG ACGAGAAAAC ACTACTTGTA TAAGTACTTT

1445 1450 1455 1460 1465 1470 1475 1480 1485 1490 1495 1500
* * * * *
TGTGGAAGTC AGTACGATCA AGTTGTTGAC TGTGATTAGA TCACATCTTA AATTCATTTT
ACACCTTCAG TCATGCTAGT TCAACAAC TGACATAATCT AGTGTAAGAT TTAAGTAAAG

1505 1510 1515 1520 1525 1530 1535 1540 1545 1550 1555 1560
* * * * *
TAGACTCAAA ACCTGGAGAT GCAGCTACTG GAATGGTGT TTGTCAGACT TCCAAATCCT
ATCTGAGTTT TGGACCTCTA CGTGGATGAC CTTACCACAA AACAGTCTGA AGGTTTAGGA

1565 1570 1575 1580 1585 1590 1595 1600 1605 1610 1615 1620
* * * * *
GGAAGGACAC AGTGATGAAT GTACTATATC TGAACATAGA AACTCGGGCT TGAGTGAGAA
CCTTCTGTG TCACTACTTA CATGATATAG ACTTGATCTT TTGAGCCCGA ACTCACTCTT

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FIG. 7 - CONT'D

1625 1630	1635 1640	1645 1650	1655 1660	1665 1670	1675 1680
GAGCTTGAC	AGCCAACGAG	ACACATTGCC	TTCTGGAGCT	GGGAGACAAA	GGAGGAATTT
CTCGAACGTG	TCGGTTGCTC	TGTGTAACGG	AAGACCTCGA	CCCTCTGTTT	CCTCCTTAA
1685 1690	1695 1700	1705 1710	1715 1720	1725 1730	1735 1740
ACTTTCTTCA	CCAAGTGCAA	TAGATTACTG	ATGTGATATT	CTGTTGCTTT	ACAGTTACAG
TGAAAGAAGT	GGTTCACGTT	ATCTAATGAC	TACACTATAA	GACAACGAAA	TGTCAATGTC
1745 1750	1755 1760	1765 1770	1775 1780	1785 1790	1795 1800
TTGATGTTTG	GGGATCGATG	TGCTCAGCCA	AATTTCTGT	TTGAAATATC	ATGTTAAATT
AACTACAAAC	CCCTAGCTAC	ACGAGTCGGT	TTAAAGGACA	AACTTTATAG	TACAATTTAA
1805 1810	1815 1820	1825 1830	1835 1840	1845 1850	1855 1860
AGAATGAATT	TATCTTTACC	AAAAACCATG	TTGCGTTCAA	AGAGGTGAAC	ATTAAATAT
TCTTACTTAA	ATAGAAATGG	TTTTTGGTAC	AACGCAAGTT	TCTCCACTTG	TAATTTTATA
1865 1870	1875 1880	1885 1890	1895 1900	1905 1910	1915 1920
AGAGACAGGA	CAGAATGTGT	TCTTTTCTCC	TCTACCAGTC	CTATTTTTC	ATGGGAAGAC
TCTCTGTCT	GTCTTACACA	AGAAAAGAGG	AGATGGTCAG	GATAAAAAGT	TACCTTCTG
1925 1930	1935 1940	1945 1950	1955 1960	1965 1970	1975 1980
TCAGGAGTCT	GCCACTTGTC	AAAGAAGGTG	CTGATCCTAA	GAATTTTTC	TTCTCAGAAT
AGTCTTCAGA	CGGTGAACAG	TTTCTTCCAC	GACTAGGATT	CTTAAAAAGT	AAGAGTCTTA
1985 1990	1995 2000	2005 2010	2015 2020	2025 2030	2035 2040
TCGGTGTGCT	GCCAACTTGA	TGTTCACCT	GCCACAAACC	ACCAGGACTG	AAAGAAGAAA
AGCCACACGA	CGGTGAACT	ACAAGGTGGA	CGGTGTTTGG	TGGTCCCTGAC	TTTCTCTTT
2045 2050	2055 2060	2065 2070	2075 2080	2085 2090	2095 2100
ACAGTACAGA	AGGCAAAGTT	TACAGATGTT	TTTAATTCTA	GTATTTTATC	TGGAACAAC
TGTCATGTCT	TCCGTTTCAA	ATGCTTACAA	AAATTAAGAT	CATAAAATAG	ACCTTGTTGA
2105 2110	2115 2120	2125 2130	2135 2140	2145 2150	2155 2160
TGTAGCAGCT	ATATATTTCC	CCTTGGTCCC	AAGCCTGATA	CTTTAGCCAT	CATAACTCAC
ACATCGTCEA	TATATAAAGG	GGAACCAGGG	TTCGGACTAT	GAAATCGGTA	GTATTGAGTG
2165 2170	2175 2180	2185 2190	2195 2200	2205 2210	2215 2220
TAACAGGGAG	AAGTAGCTAG	TAGCAATGTG	CCTTGATTGA	TTAGATAAAG	ATTTCTAGTA
ATTGTCCCTC	TTCATCGATC	ATCGTTACAC	GGAACCTAAT	AATCTATTTT	TAAAGATCAT
2225 2230	2235 2240	2245 2250	2255 2260	2265 2270	2275 2280
GGCAGCAAAA	GACCAAATCT	CAGTTGTTTG	CTTCTTGCCA	TCAGTGGTCC	AGGTCTTCAG
CCGTCGTTTT	CTGGTTTAGA	GTCAACAAAC	GAAGAACGGT	AGTGACCAGG	TOCAGAAGTC
2285 2290	2295 2300	2305 2310	2315 2320	2325 2330	2335 2340
TTTCCGAATC	TCTTTCCCTT	CCCCGTGGGT	CTATTTGTCG	TATGTGACTT	GCGCTTAATC
AAAGGCTTAG	AGAAAGGGAA	GGGGACACCA	GATAACAGCG	ATACACTGAA	CCCGAATTAG
2345 2350	2355 2360	2365 2370	2375 2380	2385 2390	2395 2400

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FIG. 7 - CONT'D

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      *           *           *           *           *
CAATATTTTG CCTTTTCTCT ATATCAAAAA ACCTTTACAG TTAGCAGGGA TGTTCCTTAC
GTTATAAAAC GGAAAAAAGA TATAGTTTTT TGGAAATGTC AATCGTCCCT ACAAGGAATG

2405 2410 2415 2420 2425 2430 2435 2440 2445 2450 2455 2460
      *           *           *           *           *
CGAGGATTTT TAACCCCCAA TCTCTCATAA TCGCTAGTGT TTAAAAGGCT AAGAATAGTG
GCTCCTAAAA ATTGGGGGTT AGAGAGTATT AGCGATCACA AATTTTCCGA TTCTTATCAC

2465 2470 2475 2480 2485 2490 2495 2500 2505 2510 2515 2520
      *           *           *           *           *
GGGCCCCAAC GATGTGGTAG GTGATAAAGA GGCATCTTTT CTAGAGACAC ATTGGACCAG
CCCGGGTTGG CTACACCATC CACTATTCTT COGTAGAAAA GATCTCTGTG TAACCTGGTC

2525 2530 2535 2540 2545 2550 2555 2560 2565 2570 2575 2580
      *           *           *           *           *
ATGAGGATCC GAAACGGCAG CCTTTACGTT CATCACCTGC TAGAACCTCT CGTAGTCCAT
TACTCCTAGG CTTTGCCGTC GGAAATGCAA GTAGTGGACG ATCTTGAGA GCATCAGGTA

2585 2590 2595 2600 2605 2610 2615 2620 2625 2630 2635 2640
      *           *           *           *           *
CACCATTCTT TGGCATTGGA ATTCTACTGG AAAAAATAC AAAAAGCAAA ACAAACCTT
GTGGTAAAGA ACCGTAACTT TAAGATGACC TTTTTTATG TTTTTCGTTT TGTTTTGGGA

2645 2650 2655 2660 2665 2670 2675 2680 2685 2690 2695 2700
      *           *           *           *           *
CAGCACTGTT ACAAGAGGCC ATTTAAGTAT CTTGTGCTTC TTCACCTACC CATTAGCCAG
GTCGTGACAA TGTTCTCCGG TAAATTCATA GAACACGAAG AAGTGAATGG GTAATCGGTC

2705 2710 2715 2720 2725 2730 2735 2740 2745 2750 2755 2760
      *           *           *           *           *
GTTCTCATTA GGTTTTGCTT GGGCCTCCCT GGCCTGAAC CTTAGGCTTT GTATGACAGT
CAAGAGTAAT CCAAAACGAA CCGGAGGGA CCGTGACTTG GAATCCGAAA CATACTGTCA

2765 2770 2775 2780 2785 2790 2795 2800 2805 2810 2815 2820
      *           *           *           *           *
GAAGCAGCAC TGTGAGTGGT TCAAGCACAC TGGATATAA AACAGTCATG GCCTGAGATG
CTTCGTGCTG ACACTCACCA AGTTCGTGTG ACCTTATATT TTGTCAGTAC CGGACTCTAC

2825 2830 2835 2840 2845 2850 2855 2860 2865 2870 2875 2880
      *           *           *           *           *
CAGGTGATGC CATTACAGAA CCAATCGTG GCACGTATTG CTGTGCTCTC TCTCAGAGTG
GTCCACTACG GPAATGTCTT GGTTTAGCAC CGTGCATAAC GACACAGAGG AGAGTCTCAC

2885 2890 2895 2900 2905 2910 2915 2920 2925 2930 2935 2940
      *           *           *           *           *
ACAGTCATAA ATACTGTCAA ACAATAAAGG GAGAATGGTG CTGTTTAAAG TCACATCCCT
TGTCAGTATT TATGACAGTT TGTATTTC CTTTACCAC GACAAATTTC AGTGTAGGGA

2945 2950 2955 2960 2965 2970 2975 2980 2985 2990 2995 3000
      *           *           *           *           *
GTAAATTGCA GAATTCAAAA GTGATTATCT CTTTGATCTA CTTGCCTCAT TTCCCTATCT
CATTTAACGT CTTAAGTTTT CACTAATAGA GAAACTAGAT GAACGGAGTA AAGGGATAGA

3005 3010 3015 3020 3025 3030 3035 3040 3045 3050 3055 3060
      *           *           *           *           *
TCTCCCCCAC GGTATCCTAA ACTTTAGACT TCCACTGTT CTGAAAGGAG ACATTGCTCT
AGAGGGGGTG CCATAGGATT TGAAATCTGA AGGGTGACAA GACTTTCCTC TGTAACGAGA

3065 3070 3075 3080 3085 3090 3095 3100 3105 3110 3115 3120
      *           *           *           *           *
ATGTCTGCCT TCGACCACAG CAAGCCATCA TCCTCCATTG CTCCGGGGA CTCAAGAGGA

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FIG. 7 - CONT'D

TACAGACGGA AGCTGGTGTC GTTCGGTAGT AGGAGGTAAC GAGGGCCCCCT GAGTTCCTCT
 3125 3130 3135 3140 3145 3150 3155 3160 3165 3170 3175 3180
 * * * * *
 ATCTGTTTCT CTGCTGTCAA CTTCCTCATCT GGCTCAGCAT AGGGTCACTT TGCCATTATG
 TAGACAAAGA GACGACAGTT GAAGGGTAGA CCGAGTCGTA TCCCAGTGAA ACGGTAATAC
 3185 3190 3195 3200 3205 3210 3215 3220 3225 3230 3235 3240
 * * * * *
 CAAATGGAGA TAAAAGCAAT TCTGGCTGTC CAGGAGCTAA TCTGACCGTT CTATTGTGTG
 GTTTACCTCT ATTTTCGTTA AGACCGACAG GTCCTCGATT AGACTGGCAA GATAACACAC
 3245 3250 3255 3260 3265 3270 3275 3280 3285 3290 3295 3300
 * * * * *
 GATGACCACA TAAGAAGGCA ATTTTAGTGT ATTAATCATA GATTATTATA AACTATAAAC
 CTACTGGTGT ATCTTCCGT TAAAATCACA TAATTAGTAT CTAATAATAT TTGATATTTG
 3305 3310 3315 3320 3325 3330 3335 3340 3345 3350 3355 3360
 * * * * *
 TTAAGGGCAA GGAGTTTATT ACAATGTATC TTTATTAAAA CAAAAGGGTG TATAGTGTTC
 AATTCCCGTT CCTCAAATAA TGTTACATAG AAATAATTTT GTTTTCCCAC ATATCACAAG
 3365 3370 3375 3380 3385 3390 3395 3400 3405 3410 3415 3420
 * * * * *
 ACAAACTGTG AAAATAGTGT AAGAACTGTA CATGTGTGAGC TCTGGTTATT TTTCTCTTGT
 TGTTTGACAC TTTTATCACA TTCTTGACAT GTAACACTCG AGACCAATAA AAAGAGAACA
 3425 3430 3435 3440 3445 3450 3455 3460 3465 3470 3475 3480
 * * * * *
 ACCATAGAAA AATGTATAAA AATTATCAAA AAGCTAATGT GCAGGGATAT TGCCTTATTT
 TGGTATCTTT TTACATATTT TTAATAGTTT TTCGATTACA CGTCCCTATA ACGGAATAAA
 3485 3490 3495 3500 3505 3510 3515 3520 3525 3530 3535 3540
 * * * * *
 GTCTGTAAAA AATGGAGCTC AGTAACATAA CTGCTTCTTG GAGCTTTGGA ATATTTTATC
 CAGACATTTT TTACCTCGAG TCATTGTATT GACGAAGAAC CTCGAAACCT TATAAAATAG
 3545 3550
 *
 CTGTATTCTT GTTT (SEQ ID NO:7)
 GACATAAGAA CAAA

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FIG. 8

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      5      10      15      20      25      30      35      40      45      50
      *      *      *      *      *      *      *      *      *      *
CTCCCAACA ATG GCG GCT CCG AGC CCG AGC GGC GGC GGC GGC TCC GGG GGC
GAGGGTTGT TAC CGC CGA GGC TCG GGC TCG CCG CCG CCG CCG AGG CCC CCG
      Met Ala Ala Pro Ser Pro Ser Gly Gly Gly Gly Ser Gly Gly>

      55      60      65      70      75      80      85      90      95
      *      *      *      *      *      *      *      *      *
GGC AGC GGC AGC GGC ACC CCC GGC CCC GTA GGG TCC CCG GCG CCA GGC
CCG TCG CCG TCG CCG TGG GGG CCG GGG CAT CCC AGG GGC CCG GGT CCG
Gly Ser Gly Ser Gly Thr Pro Gly Pro Val Gly Ser Pro Ala Pro Gly>

100      105      110      115      120      125      130      135      140      145
      *      *      *      *      *      *      *      *      *      *
CAC CCG GCC GTC AGC AGC ATG CAG GGT AAA CGC AAA GCA CTG AAG TTG
GTG GGC CGG CAG TCG TCG TAC GTC CCA TTT GCG TTT CGT GAC TTC AAC
His Pro Ala Val Ser Ser Met Gln Gly Lys Arg Lys Ala Leu Lys Leu>

150      155      160      165      170      175      180      185      190      195
      *      *      *      *      *      *      *      *      *      *
AAT TTT GCA AAT CCA CCT TTC AAA TCT ACA GCA AGG TTT ACT CTG AAT
TTA AAA CGT TTA GGT GGA AAG TTT AGA TGT CGT TCC AAA TGA GAC TTA
Asn Phe Ala Asn Pro Pro Phe Lys Ser Thr Ala Arg Phe Thr Leu Asn>

200      205      210      215      220      225      230      235      240
      *      *      *      *      *      *      *      *      *
CCC AAT CCT ACA GGA GTT CAA AAC CCA CAC ATA GAG AGA CTG AGA ACA
GGG TTA GGA TGT CCT CAA GTT TTG GGT GTG TAT CTC TCT GAC TCT TGT
Pro Asn Pro Thr Gly Val Gln Asn Pro His Ile Glu Arg Leu Arg Thr>

245      250      255      260      265      270      275      280      285      290
      *      *      *      *      *      *      *      *      *      *
CAC AGC ATT GAG TCA TCA GGA AAA CTG AAG ATC TCC CCT GAA CAA CAC
GTG TCG TAA CTC AGT AGT CCT TTT GAC TTC TAG AGG GGA CTT GTT GTG
His Ser Ile Glu Ser Ser Gly Lys Leu Lys Ile Ser Pro Glu Gln His>

295      300      305      310      315      320      325      330      335
      *      *      *      *      *      *      *      *      *
TGG GAT TTC ACT GCA GAG GAC TTG AAA GAC CTT GGA GAA ATT GGA CGA
ACC CTA AAG TGA CGT CTC CTG AAC TTT CTG GAA CCT CTT TAA CCT GCT
Trp Asp Phe Thr Ala Glu Asp Leu Lys Asp Leu Gly Glu Ile Gly Arg>

340      345      350      355      360      365      370      375      380      385
      *      *      *      *      *      *      *      *      *      *
GGA GCT TAT GGT TCT GTC AAC AAA ATG GTC CAC AAA CCA AGT GGG CAA
CCT CGA ATA CCA AGA CAG TTG TTT TAC CAG GTG TTT GGT TCA CCC GTT
Gly Ala Tyr Gly Ser Val Asn Lys Met Val His Lys Pro Ser Gly Gln>

390      395      400      405      410      415      420      425      430      435
      *      *      *      *      *      *      *      *      *      *
ATA ATG GCA GTT AAA AGA ATT CGG TCA ACA GTG GAT GAA AAA GAA CAA
TAT TAC CGT CAA TTT TCT TAA GCC AGT TGT CAC CTA CTT TTT CTT GTT
Ile Met Ala Val Lys Arg Ile Arg Ser Thr Val Asp Glu Lys Glu Gln>

440      445      450      455      460      465      470      475      480
      *      *      *      *      *      *      *      *      *
AAA CAA CTT CTT ATG GAT TTG GAT GTA GTA ATG CGG AGT AGT GAT TGC
TTT GTT GAA GAA TAC CTA AAC CTA CAT CAT TAC GCC TCA TCA CTA ACG
Lys Gln Leu Leu Met Asp Leu Asp Val Val Met Arg Ser Ser Asp Cys>

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FIG. 8 - CONT'D

485 490 495 500 505 510 515 520 525 530
 * * * * * * * *
 CCA TAC ATT GTT CAG TTT TAT GGT GCA CTC TTC AGA GAG GGT GAC TGT
 GGT ATG TAA CAA GTC AAA ATA CCA CGT GAG AAG TCT CTC CCA CTG ACA
 Pro Tyr Ile Val Gln Phe Tyr Gly Ala Leu Phe Arg Glu Gly Asp Cys>

535 540 545 550 555 560 565 570 575
 * * * * * * * *
 TGG ATC TGT ATG GAA CTC ATG TCT ACC TCG TTT GAT AAG TTT TAC AAA
 ACC TAG ACA TAC CTT GAG TAC AGA TGG AGC AAA CTA TTC AAA ATG TTT
 Trp Ile Cys Met Glu Leu Met Ser Thr Ser Phe Asp Lys Phe Tyr Lys>

580 585 590 595 600 605 610 615 620 625
 * * * * * * * *
 TAT GTA TAT AGT GTA TTA GAT GAT GTT ATT CCA GAA GAA ATT TTA GGC
 ATA CAT ATA TCA CAT AAT CTA CTA CAA TAA GGT CTT CTT TAA AAT CCG
 Tyr Val Tyr Ser Val Leu Asp Asp Val Ile Pro Glu Glu Ile Leu Gly>

630 635 640 645 650 655 660 665 670 675
 * * * * * * * *
 AAA ATC ACT TTA GCA ACT GTG AAA GCA CTA AAC CAC TTA AAA GAA AAC
 TTT TAG TGA AAT CGT TGA CAC TTT CGT GAT TTG GTG AAT TTT CTT TTG
 Lys Ile Thr Leu Ala Thr Val Lys Ala Leu Asn His Leu Lys Glu Asn>

680 685 690 695 700 705 710 715 720
 * * * * * * * *
 TTG AAA ATT ATT CAC AGA GAT ATC AAA CCT TCC AAT ATT CTT CTG GAC
 AAC TTT TAA TAA GTG TCT CTA TAG TTT GGA AGG TTA TAA GAA GAC CTG
 Leu Lys Ile Ile His Arg Asp Ile Lys Pro Ser Asn Ile Leu Leu Asp>

725 730 735 740 745 750 755 760 765 770
 * * * * * * * *
 AGA AGT GGA AAT ATT AAG CTC TGT GAC TTC GGC ATC AGT GGA CAG CTT
 TCT TCA CCT TTA TAA TTC GAG ACA CTG AAG CCG TAG TCA CCT GTC GAA
 Arg Ser Gly Asn Ile Lys Leu Cys Asp Phe Gly Ile Ser Gly Gln Leu>

775 780 785 790 795 800 805 810 815
 * * * * * * * *
 GTG GAC TCT ATT GCC AAG ACA AGA GAT GCT GGC TGT AGG CCA TAC ATG
 CAC CTG AGA TAA CCG TTC TGT TCT CTA CGA CCG ACA TCC GGT ATG TAC
 Val Asp Ser Ile Ala Lys Thr Arg Asp Ala Gly Cys Arg Pro Tyr Met>

820 825 830 835 840 845 850 855 860 865
 * * * * * * * *
 GCA CCT GAA AGA ATA GAC CCA AGC GCA TCA CGA CAA GGA TAT GAT GTC
 CGT GGA CTT TCT TAT CTG GGT TCG CGT AGT GCT GTT CCT ATA CTA CAG
 Ala Pro Glu Arg Ile Asp Pro Ser Ala Ser Arg Gln Gly Tyr Asp Val>

870 875 880 885 890 895 900 905 910 915
 * * * * * * * *
 CGC TCT GAT GTC TGG AGT TTG GGG ATC ACA TTG TAT GAG TTG GCC ACA
 GCG AGA CTA CAG ACC TCA AAC CCC TAG TGT AAC ATA CTC AAC CCG TGT
 Arg Ser Asp Val Trp Ser Leu Gly Ile Thr Leu Tyr Glu Leu Ala Thr>

920 925 930 935 940 945 950 955 960
 * * * * * * * *
 GGC CGA TTT CCT TAT CCA AAG TGG AAT AGT GTA TTT GAT CAA CTA ACA
 CCG GCT AAA GGA ATA GGT TTC ACC TTA TCA CAT AAA CTA GTT GAT TGT
 Gly Arg Phe Pro Tyr Pro Lys Trp Asn Ser Val Phe Asp Gln Leu Thr>

965 970 975 980 985 990 995 1000 1005 1010
 * * * * * * * *

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FIG. 8 - CONT'D

CAA GTC GTG AAA GGA GAT CCT CCG CAG CTG AGT AAT TCT GAG GAA AGG
 GTT CAG CAC TTT CCT CTA GGA GGC GTC GAC TCA TTA AGA CTC CTT TCC
 Gln Val Val Lys Gly Asp Pro Pro Gln Leu Ser Asn Ser Glu Glu Arg>

1015 1020 1025 1030 1035 1040 1045 1050 1055

GAA TTC TCC CCG AGT TTC ATC AAC TTT GTC AAC TTG TGC CTT ACG AAG
 CTT AAG AGG GGC TCA AAG TAG TTG AAA CAG TTG AAC ACG GAA TGC TTC
 Glu Phe Ser Pro Ser Phe Ile Asn Phe Val Asn Leu Cys Leu Thr Lys>

1060 1065 1070 1075 1080 1085 1090 1095 1100 1105

GAT GAA TCC AAA AGG CCA AAG TAT AAA GAG CTT CTG AAA CAT CCC TTT
 CTA CTT AGG TTT TCC GGT TTC ATA TTT CTC GAA GAC TTT GTA GGG AAA
 Asp Glu Ser Lys Arg Pro Lys Tyr Lys Glu Leu Leu Lys His Pro Phe>

1110 1115 1120 1125 1130 1135 1140 1145 1150 1155

ATT TTG ATG TAT GAA GAA CGT GCC GTT GAG GTC GCA TGC TAT GTT TGT
 TAA AAC TAC ATA CTT CTT GCA CGG CAA CTC CAG CGT ACG ATA CAA ACA
 Ile Leu Met Tyr Glu Glu Arg Ala Val Glu Val Ala Cys Tyr Val Cys>

1160 1165 1170 1175 1180 1185 1190 1195 1200

AAA ATC CTG GAT CAA ATG CCA GCT ACT CCC AGC TCT CCC ATG TAT GTC
 TTT TAG GAC CTA GTT TAC GGT CGA TGA GGG TCG AGA GGG TAC ATA CAG
 Lys Ile Leu Asp Gln Met Pro Ala Thr Pro Ser Ser Pro Met Tyr Val>

1205 1210 1215 1220 1225 1230 1235 1240 1245 1250 1255 1260

GAT TGAT ATCGCTGCTA CATCAGACTC TAGAAAAAAG GGCTGAGAGG AAGCAAGACG
 CTA ACTA TAGCGACGAT GTAGTCTGAG ATCTTTPTTC CCGACTCTCC TTCGTTCTGC
 Asp> (SEQ ID NO:10)

1265 1270 1275 1280 1285 1290 1295 1300 1305 1310 1315 1320

TAAAGAATTT TCATCCCGTA TCACAGTGT TTTATGCTC GCCCAGACAC CATGTGCAAT
 ATTCTTTAAA AGTAGGGCAT AGTGTACAAA AAATAACGAG CGGGTCTGTG GTACACGTTA

1325 1330 1335 1340 1345 1350 1355 1360 1365 1370 1375 1380

AAGATTGGTG TTCGTTTCCA TCATGTCTGT ATACTCCTGT CACCTAGAAC GTGCATCCTT
 TTCTAACCAC AAGCAAAGGT AGTACAGACA TATGAGGACA GTGGATCTTG CACGTAGGAA

1385 1390 1395 1400 1405 1410 1415 1420 1425 1430 1435 1440

GTAATACCTG ATTGATCACA CAGTGTAGT GCTGGTCAGA GAGACCTCAT CCTGCTCTTT
 CATTATGGAC TAACTAGTGT GTCACAATCA CGACCAGTCT CTCTGGAGTA GGACGAGAAA

1445 1450 1455 1460 1465 1470 1475 1480 1485 1490 1495 1500

TGTGATGAAC ATATTCATGA AATGTGGAAG TCAGTACGAT CAAGTTGTTG ACTGTGATTA
 AACTACTTGT TATAAGTACT TTACACCTTC AGTCATGCTA GTTCAACAAC TGACACTAAT

1505 1510 1515 1520 1525 1530 1535 1540 1545 1550 1555 1560

GATCACATCT TAAATTCATT TCTAGACTCA AAACCTGGAG ATGCAGCTAC TGAATGGTG
 CTAGTGTAGA ATTTAAGTAA AGATCTGAGT TTTGGACCTC TACGTCGATG ACCTTACCAC

1565 1570 1575 1580 1585 1590 1595 1600 1605 1610 1615 1620

TTTGTGCTAGA CTTCCAAATC CTGGAAGGAC ACAGTGATGA ATGTACTATA TCTGAACATA

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FIG. 8 - CONT'D

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AAAACAGTCT GAAGGTTTAG GACCTTCCTG TGTCACTACT TACATGATAT AGACTTGTAT
1625 1630 1635 1640 1645 1650 1655 1660 1665 1670 1675 1680
* * * * *
GAAACTCGGG CTTGAGTGAG AAGAGCTTGC ACAGCCAACG AGACACATTG CCTTCTGGAG
CTTTGAGCCC GAACTCACTC TTCTCGAACG TGTCGGTTGC TCTGTGTAAC GGAAGACCTC
1685 1690 1695 1700 1705 1710 1715 1720 1725 1730 1735 1740
* * * * *
CTGGGAGACA AAGGAGGAAT TTACTTTCTT CACCAAGTGC AATAGATTAC TGATGTGATA
GACCTCTGT TTCTCCTTA AATGAAAGAA GTGGTTCACG TTATCTAATG ACTACACTAT
1745 1750 1755 1760 1765 1770 1775 1780 1785 1790 1795 1800
* * * * *
TTCTGTGCT TTACAGTTAC AGTTGATGTT TGGGGATCGA TGTGCTCAGC CAAATTTCTT
AAGACAACGA AATGTCAATG TCAACTACAA ACCCCTAGCT ACACGAGTCG GTTTAAAGGA
1805 1810 1815 1820 1825 1830 1835 1840 1845 1850 1855 1860
* * * * *
GTTTGAAATA TCATGTTAAA TTAGAATGAA TTTATCTTTA CCAAAAACCA TGTTCGTTTC
CAAACCTTAT AGTACAATTT AATCTTACTT AAATAGAAAT GGTTTTTGGT ACAACGCAAG
1865 1870 1875 1880 1885 1890 1895 1900 1905 1910 1915 1920
* * * * *
AAAGAGGTGA ACATTAAAAT ATAGAGACAG GACAGAATGT GTTCTTTTCT CCTCTACCAG
TTTCTCCACT TGTAAATTTA TATCTCTGTC CTGTCTTACA CAAGAAAAGA GGAGATGGTC
1925 1930 1935 1940 1945 1950 1955 1960 1965 1970 1975 1980
* * * * *
TCCTATTTTT CAATGGGAAG ACTCAGGAGT CTGCCACTTG TCAAAGAAGG TGCTGATCCT
AGGATAAAAA GTTACCCTTC TGAGTCCTCA GACGGTGAAC AGTTTCTTCC ACGACTAGGA
1985 1990 1995 2000 2005 2010 2015 2020 2025 2030 2035 2040
* * * * *
AAGAATTTTT CATTCTCAGA ATTGGGTGTG CTGCCAACTT GATGTTCCAC CTGCCACAA
TTCTTAAAAA GTAAGAGTCT TAAGCCACAC GACGGTTGAA CTACAAGGTG GACGGTGTTC
2045 2050 2055 2060 2065 2070 2075 2080 2085 2090 2095 2100
* * * * *
CCACCAGGAC TGAAAGAAGA AAACAGTACA GAAGGCAAAG TTTACAGATG TTTTAAATTC
GGTGGTCCIG ACTTTCCTCT TTTGTCATGT CTTCCGTTTC AAATGCTTAC AAAAATTAAG
2105 2110 2115 2120 2125 2130 2135 2140 2145 2150 2155 2160
* * * * *
TAGTATTTTA TCTGGAACAA CTTGTAGCAG CTATATATTT CCCCTTGGTC CCAAGCCTGA
ATCATAAAAT AGACCTTGTT GAACATCGTC GATATATAAA GGGGAACCAG GGTTCGGACT
2165 2170 2175 2180 2185 2190 2195 2200 2205 2210 2215 2220
* * * * *
TACTTTAGCC ATCATAACTC ACTAACAGGG AGAAGTAGCT AGTAGCAATG TGCTTGATT
ATGAAATCGG TAGTATGAG TGATGTCCC TCTTCATCGA TCATCGTTAC ACGGAACATA
2225 2230 2235 2240 2245 2250 2255 2260 2265 2270 2275 2280
* * * * *
GATTAGATAA AGATTTCTAG TAGGCAGCAA AAGACCAAAT CTCAGTTGTT TGCTTCTTGC
CTAATCTATT TCTAAAGATC ATCCGTCGTT TCTGGTTTA GAGTCAACAA ACGAAGAAGC
2285 2290 2295 2300 2305 2310 2315 2320 2325 2330 2335 2340
* * * * *
CATCACTGGT CCAGGTCTTC AGTTTCCGAA TCTCTTTCCC TTCCCCTGTG GTCTATTGTC
GTAGTGACCA GGTCCAGAAG TCAAAGGCTT AGAGAAAGG AAGGGGACAC CAGATAACAG

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FIG. 8 - CONT'D

2345	2350	2355	2360	2365	2370	2375	2380	2385	2390	2395	2400
GCTATGTGAC	TTGCGCTTAA	TCCAATATTT	TGCCTTTTTT	CTATATCAAA	AAACCTTTAC						
CGATACACTG	AACGCGAATT	AGGTTATAAA	ACGGAAAAAA	GATATAGTTT	TTTGGAATG						
2405	2410	2415	2420	2425	2430	2435	2440	2445	2450	2455	2460
AGTTAGCAGG	GATGTTCTTT	ACCGAGGATT	TTTAACCCCC	AATCTCTCAT	AATCGCTAGT						
TCAATCGTCC	CTACAAGGAA	TGGCTCCTAA	AAATTGGGGG	TTAGAGAGTA	TTAGCGATCA						
2465	2470	2475	2480	2485	2490	2495	2500	2505	2510	2515	2520
GTTTAAAAGG	CTAAGAATAG	TGGGGCCCAA	CCGATGTGGT	AGGTGATAAA	GAGGCATCTT						
CAAAATTTTC	GATTCTTATC	ACCCCGGGTT	GGCTACACCA	TCCACTATTT	CTCCGTAGAA						
2525	2530	2535	2540	2545	2550	2555	2560	2565	2570	2575	2580
TTCTAGAGAC	ACATTGGACC	AGATGAGGAT	CCGAAACGGC	AGCCTTTACG	TTCATCACCT						
AAGATCTCTG	TGTAACCTGG	TCTACTCCTA	GGCTTTGCCG	TCCGAAATGC	AAGTAGTGGA						
2585	2590	2595	2600	2605	2610	2615	2620	2625	2630	2635	2640
GCTAGAACCT	CTCGTAGTCC	ATCACCATT	CTTGGCATTG	GAATTCTACT	GGAAAAAAT						
CGATCTTGGA	GAGCATCAGG	TAGTGGTAAA	GAACCGTAAC	CTTAAGATGA	CCTTTTTTTA						
2645	2650	2655	2660	2665	2670	2675	2680	2685	2690	2695	2700
ACAAAAAGCA	AAACAAAACC	CTCAGCACTG	TTACAAGAGG	CCATTTAAGT	ATCTTGCTGT						
TGTTTTTCGT	TTTGTTTTGG	GAGTCGTGAC	AATGTTCTCC	GGTAAATCA	TAGAACACGA						
2705	2710	2715	2720	2725	2730	2735	2740	2745	2750	2755	2760
TCTTCACTTA	CCCATTAGCC	AGGTTCTCAT	TAGGTMTTGC	TTGGGCCTCC	CTGGCACTGA						
AGAAGTGAAT	GGGTAATCGG	TCCAAGAGTA	ATCCAAAACG	AACCCGGAGG	GACCGTGACT						
2765	2770	2775	2780	2785	2790	2795	2800	2805	2810	2815	2820
ACCTTAGGCT	TTGTATGACA	GTGAAGCAGC	ACTGTGAGTG	GTTCAGCAC	ACTGGAATAT						
TGGAATCCGA	AACATACTGT	CACTTCGTGC	TGACACTCAC	CAAGTTCGTG	TGACCTTATA						
2825	2830	2835	2840	2845	2850	2855	2860	2865	2870	2875	2880
AAAACAGTCA	TGGCCTGAGA	TGCAGGTGAT	GCCATTACAG	AACCAAATCG	TGGCAGGTAT						
TTTTGTGAGT	ACCGGACTCT	ACGTCCACTA	CGGTAATGTC	TTGGTTTAGC	ACCGTGCATA						
2885	2890	2895	2900	2905	2910	2915	2920	2925	2930	2935	2940
TGCTGTGTCT	CCTCTCAGAG	TGACAGTCAT	AAATACTGTC	AAACAATAAA	GGGAGAATGG						
ACGACACAGA	GGAGAGTCTC	ACTGTCAGTA	TTTATGACAG	TTTGTTATTT	CCCTCTTACC						
2945	2950	2955	2960	2965	2970	2975	2980	2985	2990	2995	3000
TGCTGTTTAA	AGTCACATCC	CTGTAAATTG	CAGAATTCAA	AAGTGATTAT	CTCTTTGATC						
ACGACAAATT	TCAGTGTAGG	GACATTTAAC	GTCTTAAGTT	TTCACTAATA	GAGAACTAG						
3005	3010	3015	3020	3025	3030	3035	3040	3045	3050	3055	3060
TACTTGCCCTC	ATTTCCCTAT	CTTCTCCCCC	ACGGTATCCT	AAACTTTAGA	CTTCCCACTG						
ATGAACGGAG	TAAAGGGATA	GAAGAGGGGG	TGCCATAGGA	TTTGAAATCT	GAAGGGTGAC						
3065	3070	3075	3080	3085	3090	3095	3100	3105	3110	3115	3120

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FIG. 8 - CONT'D

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TTCTGAAAGG AGACATTGCT CTATGTCTGC CTTGACCAC AGCAAGCCAT CATCCTCCAT
AAGACTTTCC TCTGTAACGA GATACAGACG GAAGCTGGTG TCGTTCCGTA GTAGGAGGTA

3125 3130 3135 3140 3145 3150 3155 3160 3165 3170 3175 3180
* * * * *
TGCTCCCGGG GACTCAAGAG GAATCTGTTT CTCTGCTGTC AACTTCCCAT CTGGCTCAGC
ACGAGGGCCC CTGAGTTCTC CTTAGACAAA GAGACGACAG TTGAAGGGTA GACCGAGTCG

3185 3190 3195 3200 3205 3210 3215 3220 3225 3230 3235 3240
* * * * *
ATAGGGTCAC TTGCCATTA TGCAAATGGA GATAAAAGCA ATTCTGGCTG TCCAGGAGCT
TATCCCAAGT AAACGGTAAT ACGTTTACCT CTATTTTCGT TAAGACCGAC AGGTCCTCGA

3245 3250 3255 3260 3265 3270 3275 3280 3285 3290 3295 3300
* * * * *
AATCTGACCG TTCTATTGTG TGGATGACCA CATAAGAAGG CAATTTTAGT GTATTAATCA
TTAGACTGGC AAGATAACAC ACCTACTGGT GTATTCTTCC GTTAAATCA CATAATTAGT

3305 3310 3315 3320 3325 3330 3335 3340 3345 3350 3355 3360
* * * * *
TAGATTATTA TAAACTATAA ACTTAAGGGC AAGGAGTTTA TTACAATGTA TCTTTATTAA
ATCTAATAAT ATTTGATATT TGAATTCCTG TTCCTCAAAT AATGTTACAT AGAAATAATT

3365 3370 3375 3380 3385 3390 3395 3400 3405 3410 3415 3420
* * * * *
AACAAAAGGG TGTATAGTGT TCACAACTG TGAAAATAGT GTAAGAACTG TACATTGTGA
TTGTTTTCCT ACATATCACA AGTGTGTTGAC ACTTTTATCA CATCTTTGAC ATGTAACACT

3425 3430 3435 3440 3445 3450 3455 3460 3465 3470 3475 3480
* * * * *
GCTCTGGTGA TTTTCTCTTT GTACCATAGA AAAATGTATA AAAATTATCA AAAAGCTAAT
CGAGACCAAT AAAAAGAGAA CATGGTATCT TTTTACATAT TTTTAATAGT TTTTCGATTA

3485 3490 3495 3500 3505 3510 3515 3520 3525 3530 3535 3540
* * * * *
GTGCAGGGAT ATTGCCTTAT TTGCTGTGTA AAAATGGAGC TCAGTAACAT AACTGCTTCT
CACGTCCCTA TAACGAATA AACAGACATT TTTTACCTCG AGTCATTGTA TTGACGAAGA

3545 3550 3555 3560 3565 3570 3575
* * * * *
TGGAGCTTTG GAATATTTTA TCCTGTATTC TTGTTT (SEQ ID NO:9)
ACCTCGAAAC CTTATAAAAT AGGACATAAG AACAAA

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01078

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 14/435, 16/00; C07H 21/04; C12Q 1/68; G01N 33/53

US CL : 536/23.2; 530/387.1; 530/350; 435/6; 436/7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.2; 530/387.1; 530/350; 435/6; 436/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, WPI, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 94/24159 (NATIONAL JEWISH CENTER FOR IMMUNOLOGY AND RESPIRATORY MEDICINE) 27 October 1994, see pages 8, 16, 18, 21, 22, 28, 30, 38, 59, 60.	1, 22-37
Y	Journal Of Biological Chemistry, Volume 267, No. 36, issued 25 December 1992, Seger et al, "Human T-cell Mitogen-Activated Protein Kinases Are Related To Yeast Signal Transduction Kinases", pages 25628-25631, especially page 25630.	29
Y	Molecular And Cellular Biology, Volume 13, No. 8, issued August 1993, Wu et al, "Identification and Characterization of a New Mammalian Mitogen-Activated Protein Kinase Kinase, MKK2", pages 4539-4548, especially pages 4542 and 4543.	24, 28-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	A	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

08 MAY 1996

Date of mailing of the international search report

24 MAY 1996

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01078

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 14/435, 16/00; C07H 21/04; C12Q 1/68; G01N 33/53

US CL : 536/23.2; 530/387.1; 530/350; 435/6; 436/7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.2; 530/387.1; 530/350; 435/6; 436/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, MEDLINE, WPI, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 94/24159 (NATIONAL JEWISH CENTER FOR IMMUNOLOGY AND RESPIRATORY MEDICINE) 27 October 1994, see pages 8, 16, 18, 21, 22, 28, 30, 38, 59, 60.	1, 22-37
Y	Journal Of Biological Chemistry, Volume 267, No. 36, issued 25 December 1992, Seger et al, "Human T-cell Mitogen-Activated Protein Kinases Are Related To Yeast Signal Transduction Kinases", pages 25628-25631, especially page 25630.	29
Y	Molecular And Cellular Biology, Volume 13, No. 8, issued August 1993, Wu et al, "Identification and Characterization of a New Mammalian Mitogen-Activated Protein Kinase Kinase, MKK2", pages 4539-4548, especially pages 4542 and 4543.	24, 28-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search

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